

# Ameliorative role of flower extract of *Couroupitaguianensis* on chloramphenicol -induced hematological changes in *Mus musculus*.

DR. Laxman Landge

Department of Biology, K. J. Somaiya College of Science and Commerce, Vidyavihar, Mumbai, India.

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## ABSTRACT

Flower extract of *Couroupitaguianensis* was evaluated for its protective effect against chloramphenicol induced hematological changes like aplastic anaemia, leucocytosis, thrombocytosis, etc. The dose of chloramphenicol i. e. 500 mg/1 kg. body weight of mice 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 recorded. But the flower extract of *Couroupitaguianensis* showed significantly increase in total WBC count and some leucocytes. While decrease in haemoglobin (Hb) level, RBC count, etc. The results indicate that the flower extract of *Couroupitaguianensis* significantly reduced chloramphenicol induced toxicity in bone marrow as well as haematological parameters.

Finally It is concluded that the antioxidant potential role of flower extract of *Couroupitaguianensis* was quite good to minimise the problem of anemia and other relevant disorders due to chloramphenicol treatment.

**Keywords:** Chloramphenicol, *Couroupitaguianensis*, aplastic anaemia, haematological changes

## I. INTRODUCTION

Indiscriminate use of antibiotics in poultry industry, medicines, etc. is deleterious and as a result WHO as well as European Union have banned the use of large number of antibiotics. In the recent past, major emphasis has been laid on the use of plants with their antimicrobial, anti-inflammatory, immunomodulatory, hepato-protective, renal protective and many more properties as the most viable and practical substitute for antibiotics and other medicines that are damaging the body organs. Plant products can be used as feed additive or complementary medication.

Recently it is observed that physicians give antibiotics, pain killers like NSAIDs and others i.e. 3 - 4 types of medicines to children or adults for any kind of body pain, increased body temperature, vomiting etc. Long-term use of NSAIDs may induce bleeding that result in anaemia. Antibiotics that suppress bone marrow can lead to aplastic anaemia that occurs when the bone marrow doesn't make enough red blood cells. Chloramphenicol not only alters Hb level and RBC count, but changes leucocytes and thrombocytes count also which is mainly caused by suppression of bone marrow. Now it is rarely used in USA because of its toxicity regarding haematological parameters.

The bone marrow suppression can be decreased by the use of some phyto-medicines. They possess several non-toxic compounds that can boost the antioxidant defense mechanism in body and have protective role against tissue damage induced by several chemicals and drugs (Rege N. N., et al., 1999, Stahl W. et al., 2007, Jose J. K. et al., 2000, Kumar K. B. H. et al., 2005).

*Couroupita guianensis* Aubl. belonging to the family Lecythidaceae. It is widely cultivated for its large showy flowers and reddish-brown woody capsular fruits up to 20 cm in diameter. It is grown in Indian gardens as an ornamental tree. *C. guianensis* also called as Cannonball tree is native to South India and Malaysia. It is called as ayahuma in Ayurveda. It is used extensively as an ingredient in many preparations which cure gastritis, scabies, bleeding piles, dysentery, scorpion poison and the flowers of *C. guianensis* showed analgesic and anti-inflammatory activity and immune-modulatory activity (Geetha M. et al., 2005, Pradhan D. et al., 2008 and Farrukh Aqil, et al., 2006)

## II. MATERIALS AND METHODS

### A. Plant material and authentication

The flowers of plants collected from local region of Mumbai and the plant is authenticated by BLATTER HERBARIUM, ST. Xavier's college, Mumbai-400001, India.

### B. Preparation of plant extract

The Flower powdered was first extracted with petroleum ether (60-80°C) to remove the fatty contents and the extract was discarded. The residue was exhaustively extracted in a Soxhlet apparatus for at least 12 hours with methanol and the extract was used for experiment. The solvent from extract was removed under reduced pressure controlled temperature (40°-50°C). The yield of methanolic extract was

approximately 16/17 % w/w. The dried semisolid extract was kept in lightly closed container in refrigerator till further analysis. (Gupta Vinod H. et.al.,2012).

### C. Animals- mice

The animals used for the studies of toxicity and for efficacy were healthy Albino Swiss mice (*Mus musculus*), weighing between 30-35 gm obtained from Haffkins Institute, Parel (E), Mumbai- 400012. Under the Animal Maintenance permit Registration Number Invochem Laboratory, 226, "Gauri" Commercial Complex, Station Road, Vasai Road (E), Dist. Thane-401210; CPCSEA Registration No. 851/C/04/CPCSEA, from the ministry of Social Justice and Empowerment, Government of India. After procurement, the male and female mice were kept in same cage. The cages were provided with rice husk bedding and were cleaned daily. The house was maintained at  $28 \pm 2^\circ \text{C}$  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.

### D. Drug- chloramphenicol

Chloramphenicol was procured from Mehta Pharmaceutical Limited, 315, Janki Centre, Plot No. 29, Shah Industrial Estate, off Veera Desai road, Andheri (W), Mumbai, India. It is kept in below room temperature. Chloramphenicol is beneficial to control the growth of gram positive and gram negative bacteria, however chloramphenicol at high concentrations results in hemato-toxicity, linkage to fatal aplastic anaemia (Saba et al., 2000) and hepatotoxicity. LD<sub>50</sub> of chloramphenicol is 2300 mg/kg body weight of mouse according to Pfizer material safety data sheet, 2007.

### E. Experimental protocol

Group I (6 mice) were used as controls. Group II (6 mice) received chloramphenicol i.e. 500 mg/kg. Group III (6 mice) received 200 mg of flower extract of *Couroupita guianensis*. Group IV (6 mice) received chloramphenicol i.e. 500 mg/kg and 200 mg/kg of flower extract of *Couroupita guianensis*.

### F. 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 and 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 O. W. et. al., 1975).

### G. Statistical analysis

The obtained DATA was expressed as mean  $\pm$  SD. 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.

## III. RESULTS

Table 1 shows the effect of flower extract of *Couroupita guianensis* on hematological parameters of mice like Hb, RBC, WBC and thrombocytes count. While table 2 shows differential WBC count. Hb, RBC, lymphocytes and monocytes were observed significant increased at  $P < 0.05$ . There was significant difference in total WBC count as well as neutrophils and monocytes.

## IV. DISCUSSION

Medicinal drugs and or chemicals induced anaemia Lewis S. M. et.al., 2002, other haematological disorders, tissue injuries etc. have been known. We fed mice with sunflower oil diets for 14 days with daily administration of flower extract of *Couroupita guianensis* 200 mg/kg body weight oral dose to study the effect on haematological parameters most especially on the drug injected group and separately to the another group for comparing the variations in the blood cells and recovery due to the effect of flower extract of *Couroupita guianensis*.

In the present study it was found that the flower extract of *Couroupita guianensis* Aubl can modulate the haematological variations induced by chloramphenicol. It was observed that the treatment of flower extract of *Couroupita guianensis* 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 red blood cells counts, Hb level were significantly higher in the *Couroupita guianensis* Aubl. feed groups and compared these values with groups control, II and IV. This suggests that the flower extract contain agents that induced the production of red blood cells or enhances erythropoiesis by bone the marrow. Although, the mechanisms involved were not studied. Red blood cells carry oxygen to

various parts of the body by haemoglobin (N.EUdonwa, 2009).Haemoglobin content in the *Couroupita guianensis* Aubl.fed group was significantly higher. This increase may arise as a result of increase RBC count and possibly the mineral content of the flower extract especially iron content, an increase in iron supply is necessary to elevate the haemoglobin content. This suggests that the flower may be useful in management of anaemic conditions, supporting the acclaimed folkloric usage that *Couroupita guianensis* relieves conditions of malaria and typhoid.

The haematocrit of the extract fed mice were significantly higher compared with other groups. The measure of haematocrit is the number and size of red blood cells (Porth M. C.et.al., 2002). Therestult suggests an increase in volume of RBC in flower extract and other groups is one of inability to synthesise new DNA bases or defect in cell division seen in cytotoxic drugs or haematological disease in the marrow such as myedysplasia (Craig J. O, et.al., 2006). *Couroupita guianensis* flower extract fed groups showed increased platelet count as compare to groups I, II and IV. It was observed that groups III and IV are closely related in the production of red blood cells and platelets. It is recognised that several types of anaemia are associated with arise in platelets disorders, difficiency of iron or vitamin B12 or folic acid in diet. Increase in total WBC count may be beneficial as they are vital in the body’s defence mechanism (Gilani A. H, et.al., 2000).

The chloramphenicol with flower extract of *Couroupita guianensis* group exhibited mark changes in the haematological parameters indicating that when chloramphenicol with extract diet fed mice has been ameliorated. Scientists continue to investigate the use of free radical scavengers to protect against cell injury during periods when protective cellular mechanisms are impaired (S .Faiz,, et.al., 1992). Antioxidants are involved in the prevention of free radical cellular damage, the common pathway of a variety of diseases. The subdued effect of free radicals in the chloramphenicol with *Couroupita guianensis* group must be due to the presence of such radical scavengers as important for *Couroupita guianensis* medicinal values. These antioxidants in the *Couroupita guianensis* act as defense system against free radical damage.

**TABLE 1**  
**Haematological observations after treatment and recovery with the help of flower extract of *Couroupita guianensis* in mice.**

Groups	Hbgm%	RBC/cmm×10 <sup>6</sup>	TLC×10 <sup>3</sup>	PLT ×10 <sup>5</sup>
I – Control	14.58± 0.55	6.5 ± 0.18	8.55 ± 1.11	7.68 ± 0.79
II- chloram low	14.22 ± 0.9	6.4 ± 0.48	7.78 ± 1.13	7.26 ± 1.37
III- FE of CG	16.36 ± 0.93	7.47 ± 1.02	8.7 ± 1.44	7.92 ± 0.95
IV- chloram low+ FE of CG	15.65 ± 0.8	7.4 ± 0.30	11.2 ± 1.25	7.47 ± 0.91

P values < 0.05 by ‘f’ test.

The values are expressed as Mean ± SE from 6 rats in each group.

FE of CG means flower extract of *Couroupitaguianensis*.

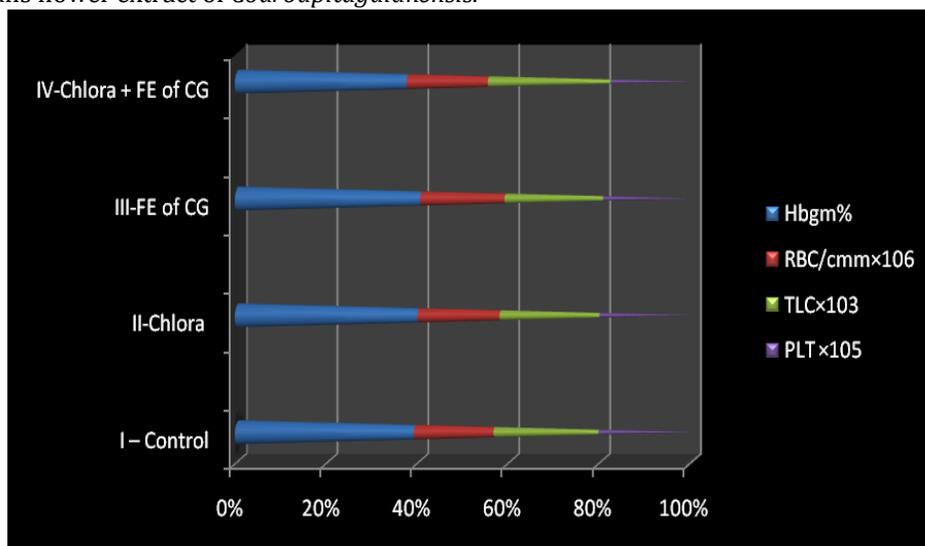


TABLE 2

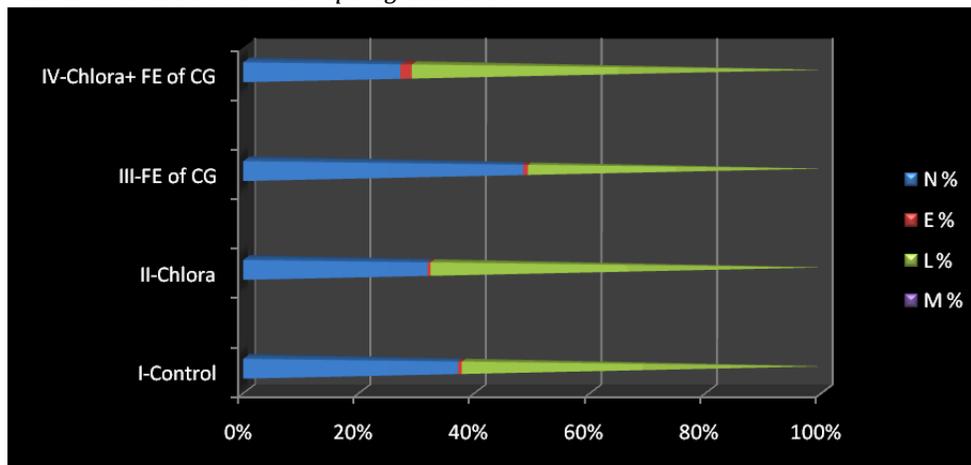
Differential WBC observations after treatment and recovery with the help of flower extract of *Couroupita guianensis* in mice.

Groups	N %	E %	L %	M %
I- Control	36.83 ± 5.47	0.66 ± 0.48	61 ± 6.1	1.16 ± 0.37
II- chloram low	31.83 ± 5.43	0.5 ± 0.5	67 ± 5.25	1 ± 0
III- FE of CG	48.16 ± 11.27	0.83 ± 0.5	50 ± 17.7	1 ± 0.9
IV- chloram low+ FE of CG	27 ± 3.83	2 ± 1.41	70 ± 3.8	0.83 ± 0.37

P values < 0.05 by 'f' test.

The values are expressed as Mean ± SE from 6 rats in each group.

FE of CG means flower extract of *Couroupita guianensis*.



## V.CONCLUSION

In conclusion, chloramphenicol caused declined the values of haemoglobin, RBC's, total leucocyte count, blood platelets, neutrophils, eosinophils, etc. The extract of *Couroupita guianensis* have the flavonoids (swietenine, sapropterin, usnic acid, lupeol and gamma tocopherol) which scavenged the free radicals that are formed due to toxicity of antibiotics or other toxic substances. Hence these flavonoids recovered the values of HB, RBC's, TLC, lymphocytes etc.

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