

## HAEMATOLOGICAL AND HISTOPATHOLOGICAL STUDIES ON RABBITS ON "MADAM CATHERINE" BLOOD TONIC

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### ABSTRACT

*Haematological and histopathological studies were conducted on a locally prepared blood tonic, named "Madam Catherine", using rabbits as experimental animals. A progressive gain in weight was observed in both animals on control and test diets. Haematological studies revealed the drug to be haematinic and non-toxic. Histopathological studies on organs also showed no evidence of hepatic or renal toxicity, jejunal atrophy or gastritis.*

**Keywords:** Blood tonic, haematology, histopathology

### INTRODUCTION

Blood tonic is a liquid preparation, which has haematinic properties. There are a number of locally prepared blood tonics on the Ghanaian market. Examples are "Durof", prepared by Dumex Pharmacy, Accra, "Dr. Asiana" blood tonic prepared by Mr. Asiana and Co., Kumasi, and "Madam Catherine" blood tonic prepared by Tawheed Laboratories, Kumasi, and others. Minamino compound is an imported blood tonic. A number of small scale industries have gone into the production of these blood tonics for varied reasons, being for economic purposes, to provide employment and to compete with imported blood tonics which are expensive and cannot be afforded by the average Ghanaian and lastly, the availability of raw materials in large quantities for the preparation of such blood tonics. These blood tonics are prepared from herbs and other materials and have reasonable prices. Unfortunately, the potency, probable toxicity and chemical composition of such traditional preparations are generally not known, but the market for them is so large because of the low standard of living of the Ghanaian population. Such low standards of living produce high incidence of nutritional anaemia among children, females, expectant and lactating mothers, because of their high iron requirements, and need for protein-rich foods.

This research work was therefore carried out to investigate the effect of "Madam Catherine" blood tonic on the blood and organs of rabbits by performing haematological and histopathological studies.

### MATERIALS AND METHODS

#### Collection of Samples

Thirty-six (36) bottles of freshly prepared "Madam Catherine" blood tonic were bought from a van selling the drug in Kumasi and were kept until needed. 20 male albino rabbits were purchased from various animal farms in Kumasi.

### Method

The rabbits were divided into groups of four, five rabbits in each group and housed individually in metal cages. A basal diet used for feeding the rabbits was made from fresh leaves and stems of *Panicum maximum* (1 part), and *Aspilia africana* (4 part). The proximate composition of the basal diet was determined.

**Table 1: Proximate Composition of Basal Diet**

Moisture	86.9%± 0.24
Crude protein	7.32%±0.0
Ash	1.05%±0.005
Crude fat	0.39%±0.02
Nitrogen free extract and fiber	4.35%

The animals were acclimatised for 7 days on the basal diet after which three graded dosages (Table 2) of "Madam Catherine" blood tonic were administered based on the dosage prescribed by the manufacturer for adult humans, (70kg body weight) which is two tablespoons full three times daily. The dosages given to the rabbits were therefore calculated on per kilogram body weight basis.

**Table 2: Dosage of "Madam Catherine" Blood Tonic Used at a Time per Rabbit and the Mean % Weight Increase of Animal**

Animal	Dosage of Madam Catherine	Mean % Increase in Body Weight
Control	0.6ml water	24.04*±5.11
Standard (Full dosage)	0.6ml blood tonic	18.05*±11.05
Half dosage	0.3ml blood tonic	18.23*±6.79
Double dosage	1.2ml blood tonic	18.32*±11.85

\*The weight increases are not statistically different at  $P>0.01$ .

The four groups of animals were raised for 84 days, and the blood tonic was administered three times a day, till the end of the study. The animals were weighed weekly; blood was drawn for haematological studies at the beginning of the study and after 21 days, 42 days, and at the end of the study. At the end of the study period, the animals were slaughtered and the following organs removed for histopathological studies: the viscera, brain, skin, eye, testes, skeletal muscle and bone.

A pathologist studied the organs in situ before they were removed. Under the haematological studies, the following parameters were determined: haemoglobin content by the cyanomethemoglobin method [1]; total red blood cell (rbc) count (by visual means, [2]); packed cell volume (PCV) by a micro-method using haematocrit tube [2] and total white blood cell (wbc) count by electronic method [2]. For histopathological studies, sections were taken, processed and

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stained by the Haematoxylin-Eosin method. About the middle of the study, a veterinary doctor who came to inspect the animals detected that the animals were heavily infested with pests (lice) and so the animals were treated. According to the doctor the infestation could cause a sharp drop in haemoglobin level.

#### Statistical Analyses

A completely randomised design was used to study the effect of the blood tonic on the blood and organs of experimental animals. Significant differences among means of the parameters were determined by carrying out analysis of variance [3].

#### RESULTS AND DISCUSSION

The effect of "Madam Catherine" blood tonic on the body, blood and organs was studied using a completely randomised design. Increase in body weight over the entire period of study is shown in Table 2.

Analysis of variance of a completely randomised design did not reveal any significant effect on body weight of animals. This observation is expected since the blood tonic, which is supposed to be haematinic, does not contain many calories, which may cause increase in body weight. Moreover, these haematinic compounds when absorbed use up certain amino acids within mucosal cells of the gut or become associated with apoferritin which is an intracellular acceptor protein for the synthesis of haemoglobin, to be incorporated into red blood cells [4]. These protein and amino acid components of the body, which may be used up to provide calories and hence increase in body weight, are withdrawn for synthesis of haemoglobin. Moreover the heavy infestation with pest might have contributed to the low gain in body weight.

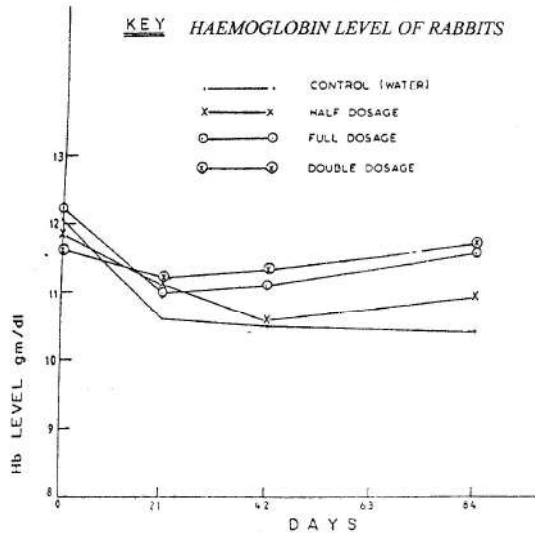


Fig. 1: Effect of mad catherine blood tonic on haemoglobin (Hb) level

The effect of "Madam Catherine" blood tonic on haemoglobin (Hb) content was investigated. Fig. 1 shows the variation of haemoglobin levels with time. In all the four groups of animals, there was an initial sharp drop in haemoglobin levels in the first three weeks of administration of the tonic, followed by a gradual increase in haemoglobin level in the three groups of animals. Analysis of variance of a completely randomised design revealed that there was a significant drop ( $P < 0.01$ ) in haemoglobin levels of animals on water and half dosage of the tonic, and full dosage but no significant drop in haemoglobin level was produced in the animals on double dosage, but rather remained fairly constant over the study period. During the study, the animals were infested with pests, which fed on the skin and blood. The reduction in haemoglobin level during this period could be attributed to the infestation. It is interesting to note that, with animals on the double dosage of the blood tonic, though they were also equally infested with the pests, maintained fairly constant haemoglobin levels (Fig. 1). This observation shows that, "Madam Catherine" blood tonic is truly haematinic. With the other three groups of animals, which showed drops in haemoglobin levels statistical analysis revealed the following differences ( $P < 0.01$ ) in this order: animals on control, produced the control - largest drop in haemoglobin level, followed by animals on half dosage, with those on full dosage showing the least drop in haemoglobin level. These results show that, without the infestation, the Hb levels would have increased significantly, with the control having the lowest increase, followed by animals on half dosage, of the tonic and then those on full dosage, with the highest increase in haemoglobin level occurring in animals on double dosage.

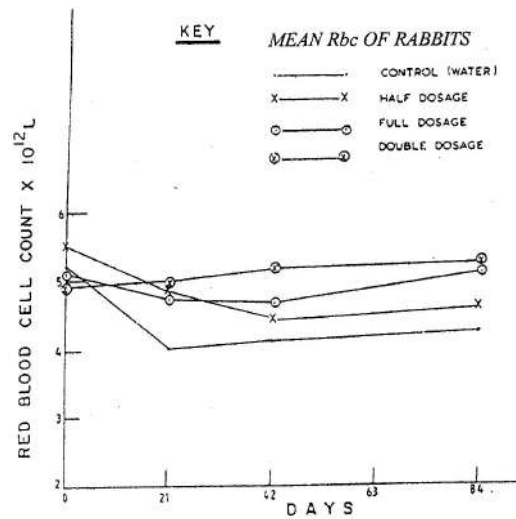


Fig. 2: Effect of mad catherine blood tonic on red blood cell (Rbc) count

The effect of "Madam Catherine" blood tonic on red blood cell (rbc) count was also investigated. Fig. 2 shows the variation of rbc count with time. Analysis of variance of a completely randomised design showed that, the blood tonic

has an effect on rbc count. With the incidence of infestation, animals on control and half dosage of the blood tonic show significant ( $P < 0.05$ ) decreases in rbc count but animals on full and double dosage of the blood tonic showed slight increases even with the infestation. This showed that, the blood tonic contains certain factors, which either regulate the synthesis of red blood cells or prolong their life span. The mean rbc count for human adults is  $5.5 \times 10^{12}/l$  [2].

The effect of "Madam Catherine" blood tonic on packed cell volume (Haematocrit value) was also assessed. Haematocrit is the determination of the volume of a given unit of blood that is composed of red cells (erythrocytes and reticulocytes). Normally, there is a direct relationship

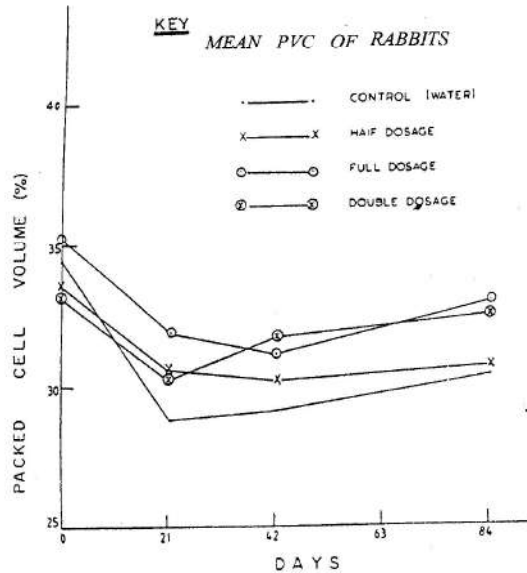


Fig. 3: Effect of mad catherine blood tonic on packed cell volume (PCV)

between the red blood cell and haematocrit value: an increase or decrease in one produces an increase or decrease in the other respectively. Fig. 3 shows that the effect of the blood tonic on PCV followed the same pattern as the rbc count. The animals on double dosage of blood tonic showed no drop in PCV, but remained within the normal levels for adults. The normal PCV levels of adult males are 44%. Thompson and Proctor [4] reported that, in certain pathological conditions this relationship does not exist. If the red blood cells are larger than normal and the haemoglobin content is greater than normal; the PCV value is greater than that which normally corresponds to that rbc count. If the red blood cells are smaller than normal and the haemoglobin level is lower than normal, the PCV value is lower than the normally expected corresponding rbc count. Figs. 2 and 3 show that, this relationship between rbc and PCV exists, and hence no pathological condition of the blood upon administration of the "Madam Catherine" blood tonic was observed for the period of study.

The effect of the blood tonic on white blood cell (wbc) count was studied using the same experimental design.

Fig. 4 shows the effect of "Madam Catherine" blood tonic on wbc count. These graphs show that, wbc count remained fairly constant with time. Analysis of variance of a completely randomised design revealed that, the preparation administered did not have any significant effect on wbc count. The average wbc count for adults is  $7.5 \times 10^9/l$  [1]. Generally, a blood tonic is not supposed to alter white blood cell count. White blood cell count is altered in pathological conditions including pathological lymphocytosis, that is increased number when there is the incidence of infection or leukemia, and leucopenia, that is reduced numbers when there is the incidence of certain types of viral infections. Since the wbc count remained fairly constant over the study period, it implies that, the blood tonic did not have a significant effect

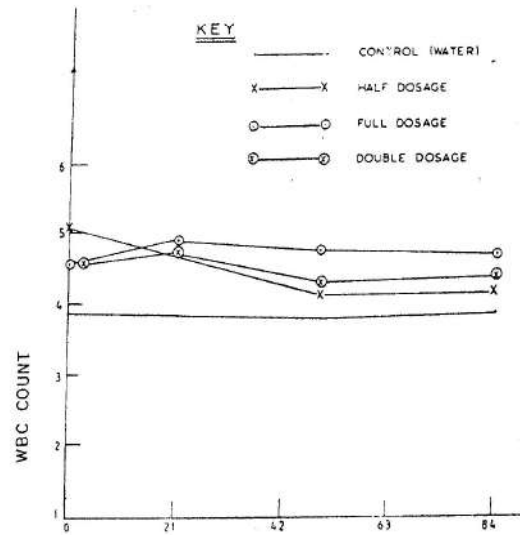


Fig. 4: Effect of mad catherine blood tonic on white blood cell (WBC) count

on wbc count and hence no evidence of disease conditions. The non-significant drops in wbc count which were observed in some of the animals could be attributed to the infestation of the pests.

#### HISTOPATHOLOGICAL STUDIES

Histopathological studies were performed on the organs of the animals after they had been slaughtered. The organs were of normal size for the rabbits examined. Attention was paid particularly to the liver, kidneys, heart, stomach and jejunum. The eye and testes were also examined. In all the specimens examined there was no change from rabbit 1 to 20. There was no evidence of renal or hepatic toxicity, or jejunal atrophy or gastritis. The blood tonic therefore did not have any toxic effect on the animals.

#### CONCLUSION

From the above discussion, the drug, administered may be said to be haematonic and non-toxic for the period of study. The investigation is continuing.

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