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HEMATOPOIETIC STUDY OF THE METHANOLIC ROOT EXTRACT OF *BETA VULGARIS* ON ALBINO RATS-AN *IN VIVO* STUDY

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ABSTRACT

Medicines play an important role in human health cure. The plant kingdom constitutes a source of new chemical compounds which may important owing to their potential use in medicine and other applications. The present study investigates the hematopoietic activity of *Beta vulgaris* in experimental rat models treated with various concentrations of the methanolic extract of the root of *Beta vulgaris.* Rats were divided into four groups of five each and the groups were as follows: Group 1- Control group; Group 2- Rats were received 100 mg/kg b.w. *Beta vulgaris* methanolic extract orally for sixteen days; Group 3- Rats were received 200 mg/kg *Beta vulgaris* methanolic extract orally for sixteen days; Group 4- Rats were received 400mg/kg *Beta vulgaris* methanolic extract orally for sixteen days. All the groups were given *Beta vulgaris* extract for sixteen days at 24 hour interval. From the Phytochemical studies it was proved that powdered root of *Beta vulgaris* contains alkaloids, flavonoids, phenols etc., In this study rats treated with methanolic root extract of *Beta vulgaris* recorded significant increase s in Packed Cell Volume (PCV), Hemoglobin concentration, Red Blood Cell counts (RBCs), and Total lymphocyte count .The other parameters such as MCV, MCH, MCHC also showed a dose dependent fashion.

KEY WORDS: ***Beta vulgaris*, Mean corpuscular volume (MCV), Mean corpuscular Hemoglobin concentration (MCHC), Mean corpuscular hemoglobin (MCH).**

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INTRODUCTION

In different parts of the world especially in Africa and Asia with high incidence of the disease, the people have learnt to manage the problem using plants which are God's gift of nature. Crude extracts from plants have been used in treating an array of diseases since ancient times although, the bioactive components of such plants remain largely unknown. Various advances in scientific research on the use of plants and herbs brought the beneficial aspects of traditional medicine and the rational for their uses to the limelight [1]. Different beetroot compounds, e.g. betalains, became especially important for phytomedicine: betalains (betacyanins and betaxanthines) have been detected only in red- violet, orange and yellow pigmented botanical species belonging to closely related families of the order caryophyllalles [2]. Betalain pigments have specifically been shown to possess various antioxidant functions [3]. Anemia in association with iron deficiency has serious implications in terms of increased morbidity and mortality rates in vulnerable groups, impaired growth and cognitive abilities in children, and reduced capacity and poor obstetric performance in adults [4]. Although many causes of anemia have been identified worldwide [5], it is agreed that nutritional deficiency due primarily to low bioavailability of dietary iron accounts for more than half the total number of cases [6]. Hemoglobin determination is one of the most convenient screening methods in anemia. Of the iron-status indicators, serum ferritin (SF) and erythrocyte protoporphyrin (EP) are among the reliable indexes available for assessing nutrition in population groups [7].Beetroot is one of the richest sources of folate, which is important for a healthy heart and for women trying to conceive, as it helps prevent spinal cord defects in the baby. Beetroot is also a source of fiber, potassium, manganese, iron, vitamin C, and a number of other vitamins and minerals. [8]The aim of the present study is to identify the hematopoietic activity of methanolic extract of *beta vulgaris* in experimental rat models.

MATERIALS AND METHODS

PLANT MATERIAL

Fresh root of *Beta vulgaris* was collected from the local market of Coimbatore. The roots were washed thoroughly with distilled water.

PREPARATION OF EXTRACT

The *Beta vulgaris* roots were cut into small pieces. It was dried at room temperature and protected from sun light and dust. Dried pieces were then powdered using laboratory homogenizer. A total 300g of the ground powder was soaked in 500ml of methanol and kept in a shaker for 48 hours at room temperature. The mixture was filtered into 500ml conical flask with wattman filter paper (No.1). The filtrate was dried at room temperature for 72 hours to produce a gel like extract which weighed 25g. Appropriate concentrations of the extract where then subsequently made by dilution with distilled water into 100mg/kg, 200mg/kg and 400mg/kg respectively and administered to the animals.

EXPERIMENTAL ANIMALS- HANDLING AND TREATMENT

A total of 20 male adult albino rats weighing between 150 – 200g were obtained from the animal house of K.M.C.H Pharmacy College, Coimbatore. The institutional ethics committee approved the experimental protocols. All the animals used in this study were placed in stainless steel cages in an air conditioned room and 12 hour dark and light schedule. Throughout the experiment, the animals were prevailed standard food pellet and water *ad libitum.* Essential cleanliness conditions were also maintained. All the animals were anaesthetized with chloroform vapors, 24 hour after last administration and blood was collected by ocular bleeding. Blood samples

were collected into EDTA treated sample bottles. The whole blood collected was then used for hematological indices. The assays were carried out in the clinical laboratory of Kovai Medical Research Centre and Hospital, Coimbatore.

DETERMINATION OF HEMATOLOGICAL INDICES

The blood sample collected into EDTA sequestrene bottles were immediately used for determination of hematological indices within 24 hours of sample collection. Determination of PCV was carried out using the Hematocrit method as described by [9, 11], Hemoglobin concentration was determined by using the cyanomethaemoglobin method [10]. The total white blood cells (WBC), White blood cell differentials, Red blood Cell (RBC) and the platelet counts were estimated using the improved Neubauer counting chamber [10, 11].

STATISTICAL ANALYSIS

Statistical analysis were carried out using window SPSS. One way analysis of variance (ANOVA) was adopted for comparison, and results were subjected to post hoc test to evaluate the significance of the difference between the mean and the measured parameters of the respective test and control group. A significant change was considered acceptable at $p<0.05$. Results of the biochemical estimations were expressed as mean±SD. The comparisons for ANOVA were done as follows;

a: comparison of GROUP 1and GROUP 2.
b: comparison of GROUP 1 and GROUP 3.
c: comparison of GROUP 1 and GROUP 4.

HEAMATOPOIETIC ACTIVTY

The effects of various doses of the methanolic extract of *Beta vulgaris* are obtained after analysis. The results of this study showed that the methanolic extract of *Beta vulgaris* increases the hematopoietic indices in a dose dependent fashion.

PACKED CELL VOLUME

Increasing the dosage concentration of Beta *vulgaris* extract had an effect of increasing the packed cell volume in all the treatment groups. Even PCV values at 100mg/kg, 24±1 percent was significantly different from that of the control group 28.05±0.310 percent. At high doses of the extract, 200 mg/kg and 400mg/kg, there was a significant increase from 29.3±0.572 percent to 38.5±0.577 percent respectively ($p<0.001$& $p<0.01$).

HEMOGLOBIN CONCENTRATION

Increase in hemoglobin concentration occurred with increased concentration of applied extract. The hemoglobin value of 12.25±0.5 g/dl at 100mg/kg increased to 13.6±0.216 g/dL at 200mg/kg and 15.25±0.310g/dL at 400mg/kg respectively. All these values are significantly different from the control group. Significant change in hemoglobin concentration also occurred between the two high doses that is 200 and 400mg/kg.

RED CELL COUNT

Animals that received 100 and 200mg/kg body weight of the extract had significantly lower red cell value than those which received 400 mg/kg weight of the extract. There was a significant difference ($p<0.05$) between the red cell count of those that received 100mg/kg dose of extract 6.272±0.098 M/µL and the normal saline in the control group 5.55±0.1 M/µL.

MCV, MCH AND MCHC

The mean Corpuscular hemoglobin (MCH) rises in test groups in a dose related fashion. The increase in MCH in group 2 was significantly different from group 1 (control). Also group-3 value, 18.175±0.45pg and group-4 value, 20.025±0.928pg were significantly higher than group-1 (control).Mean Corpuscular hemoglobin concentration (MCHC) in both the control and

test groups were statistically significant. Similarly, the mean corpuscular volume (MCV) was marginally higher in group-2, 52.5±0.955fL than group-1 value, 51±0.721fL,but the increase in group-3 value,55.275±5.095 fL, and group-4, 58.675±1.939 fL were significantly higher than group -1.

PLATELETS

The platelets count was increased in a dose dependent fashion. The group-1 value, 441±1 k/µL raised to 504.25±13.048 k/µL at 100mg/kg of extract. The groups receiving extract at a dose of 200mg/kg and 400mg/kg showed a dose dependent pattern of increase in the platelet count. Group 3 showed a value of 556±5.477 k/µL where as the group 4 value raised to 707±25.019 k/µL.

TOTAL LYMPHOCYTE VOLUME

The total lymphocyte volume was found to increase with increase in extract. The control group showed a TLC count of 5.186±0.382 k/µL, which was found to increase to 18.775±0.861 k/µL on the groups gavaged with the *Beta vulgaris* extract at a dose of 400mg/kg.

TABLE.2
HAEMATOLOGICAL INDICES OF CONTROL AND OF TEST RATS GAVAGED WITH METHANOLIC EXTRACT OF BETA VULGARIS

PARAMETERS	Group I CONTROL	LOW DOSE	MEDIUM DOSE	HIGH DOSE
RBC [M/µL]	5.55±0.1	6.272±0.098^{a*}	6.727±0.098^{b**}	7.965±0.107^{c***}
Hb [g/Dl]	11.4±0.3	12.25±0.5^{a*}	13.6±0.216^{b**}	15.25±0.310^{c***}
PCV[%]	24±1	28.05±0.310^{a*}	29.3±0.572^{b**}	38.5±0.577^{c***}
PLT [k/µL]	441±1	504.25±13.048^{a*}	556±5.477^{b**}	707±25.019^{c***}
MCV[fL]	51±0.721	52.275±5.095^{a*}	55.5±0.955^{b*}	58.675±1.939^{c**}
MCH [pg]	14.5±0.5	16.45±0.741^{a*}	18.175±0.457^{b*}	20.025±0.928^{c**}
MCHC [g/dL]	21±1	26.475±1.201^{a**}	28.2±0.258^{b*}	35.475±0.464^{c***}
TLC [k/µL]	5.186±0.382	8.15±1.112^{a*}	13.825±0.403^{b*}	18.775±0.861^{c***}
Lymphocyte[%]	67±3	71±2.828^{ans}	73.75±2.629^{b*}	86.75±2.36^{c***}
Polymorphs[%]	33±3	29±2.83^{ans}	26.25±2.629 ^{bns}	13.25±2.36^{cns}

******= significantly different from group 1(p<0.001),**=significantly different from group 1(p<0.01),*=significantly different from group 1(p<0.05) a=comparison between group 1 and group 2: b=comparison between group 1 and group 3: c= comparison between group 1 and group 4.group 1-control, group 2-received 100mg/kg of Beta vulgaris, group 3- received 200mg/kg of Beta vulgaris, group 4-received 400mg/kg of Beta vulgaris.***

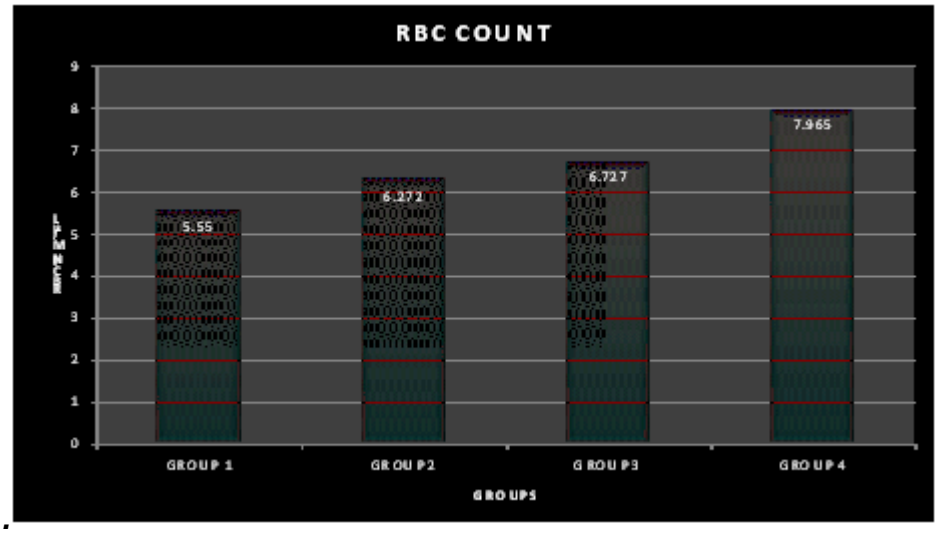

FIGURE 1
RBC COUNT CHANGES IN CONTROL AND TEST ANIMALS DURING Beta vulgaris EXTRACT TREATMENT OF RATS.

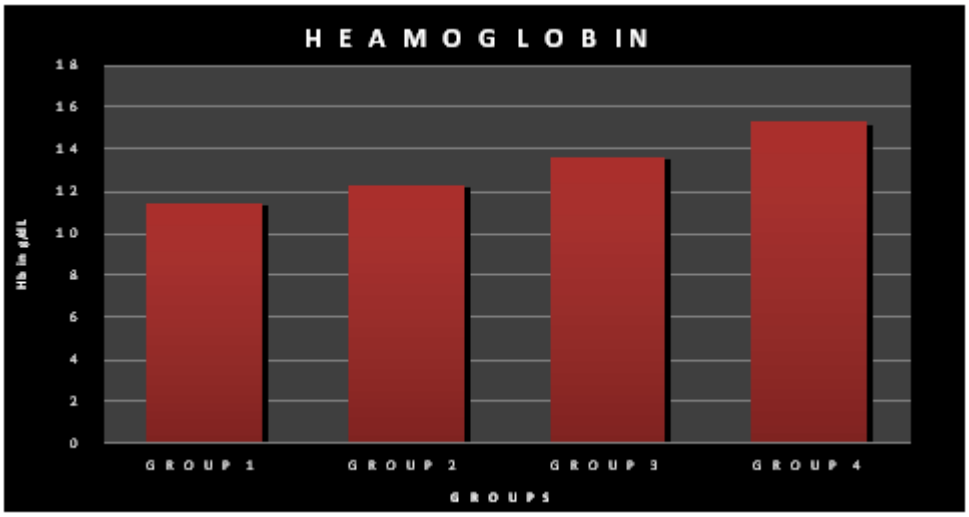

FIGURE 2
HEAMOGLOBIN CHANGES IN CONTROL AND TEST ANIMALS DURING Beta vulgaris EXTRACT TREATMENT OF RATS.

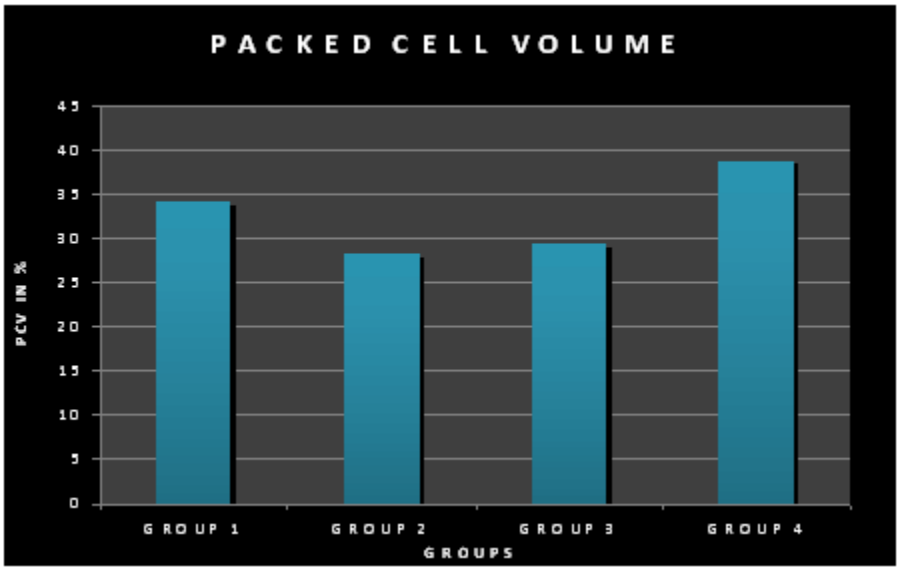

FIGURE 3
CHANGE IN PCV IN CONTROL AND TEST ANIMALS DURING Beta vulgaris EXTRACT TREATMENT OF RATS

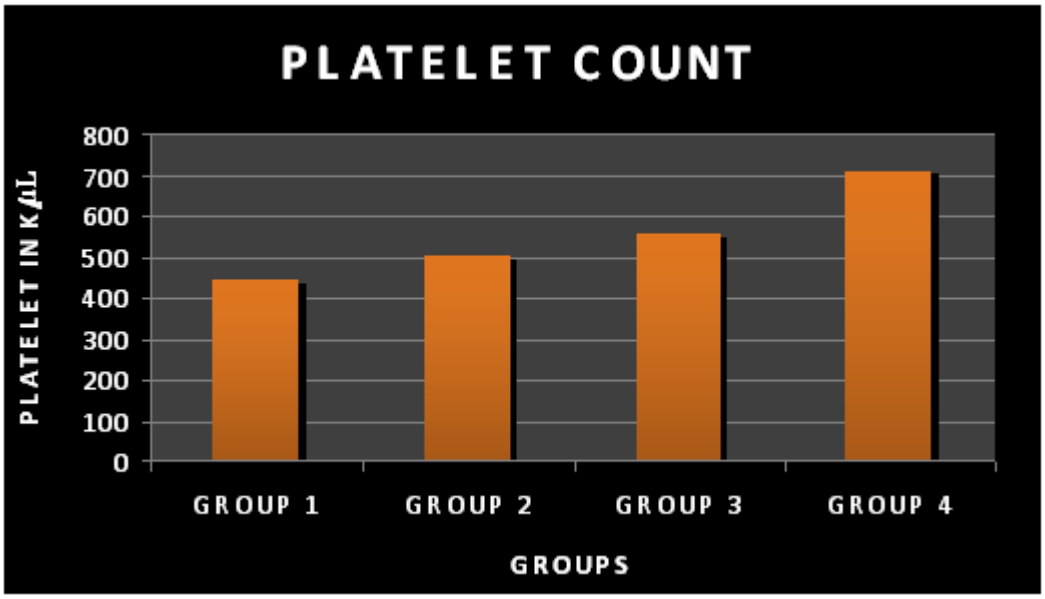

FIGURE4
CHANGE IN PLATELET COUNT IN CONTROL AND TEST ANIMALS DURING Beta vulgaris EXTRACT TREATMENT OF RATS

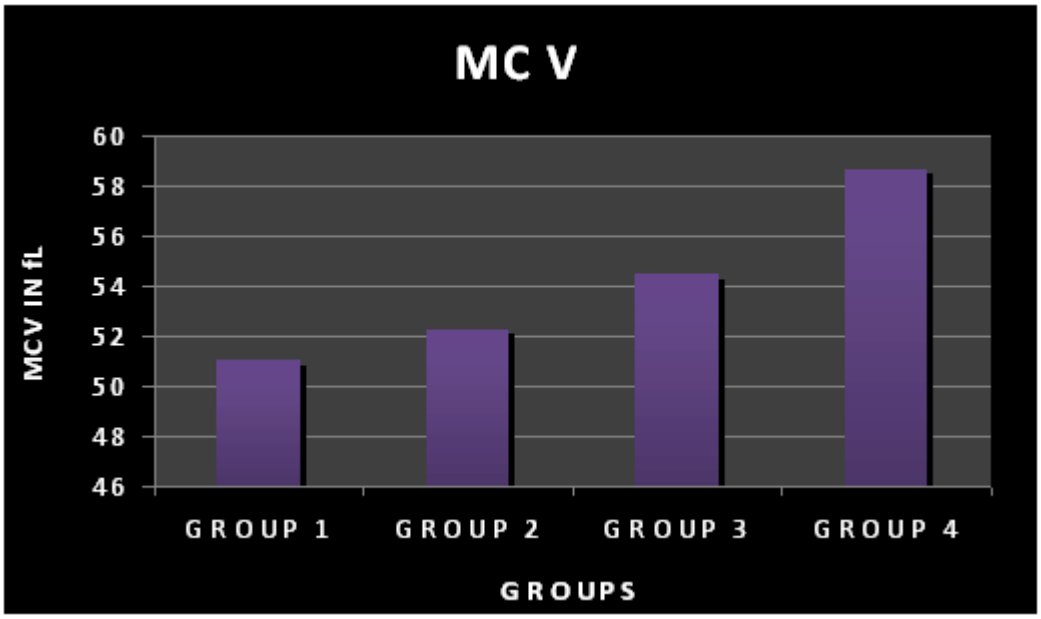

FIGURE 5
CHANGE IN MCV COUNT IN CONTROL AND TEST ANIMALS DURING Beta vulgaris EXTRACT TREATMENT OF RATS.

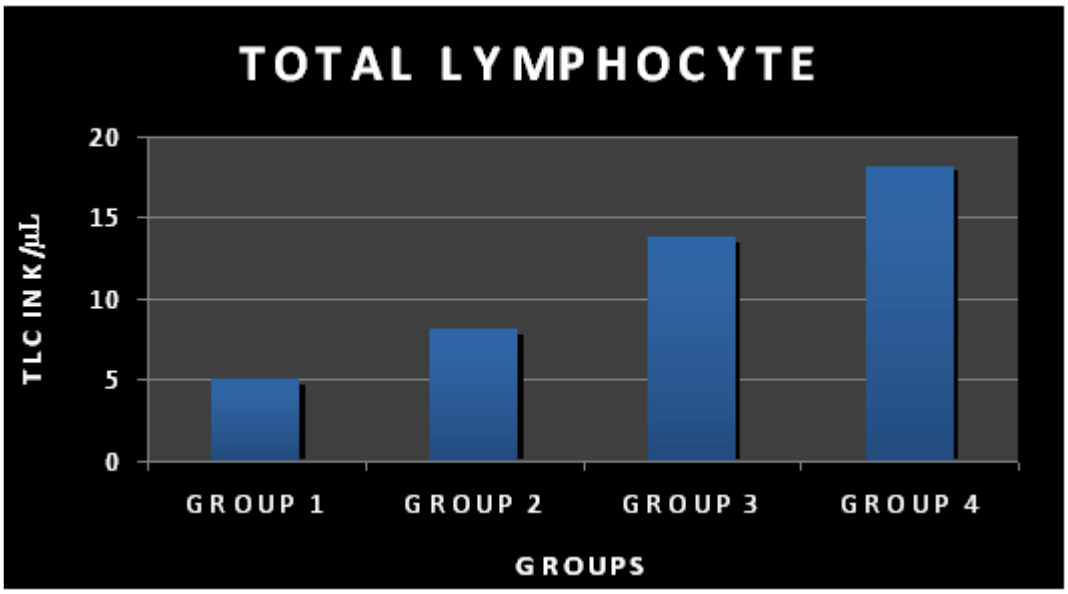

FIGURE 6
CHANGE IN TOTAL LYMPHOCYTE COUNT IN CONTROL AND TEST ANIMALS DURING Beta Vulgaris EXTRACT TREATMENT OF RATS.

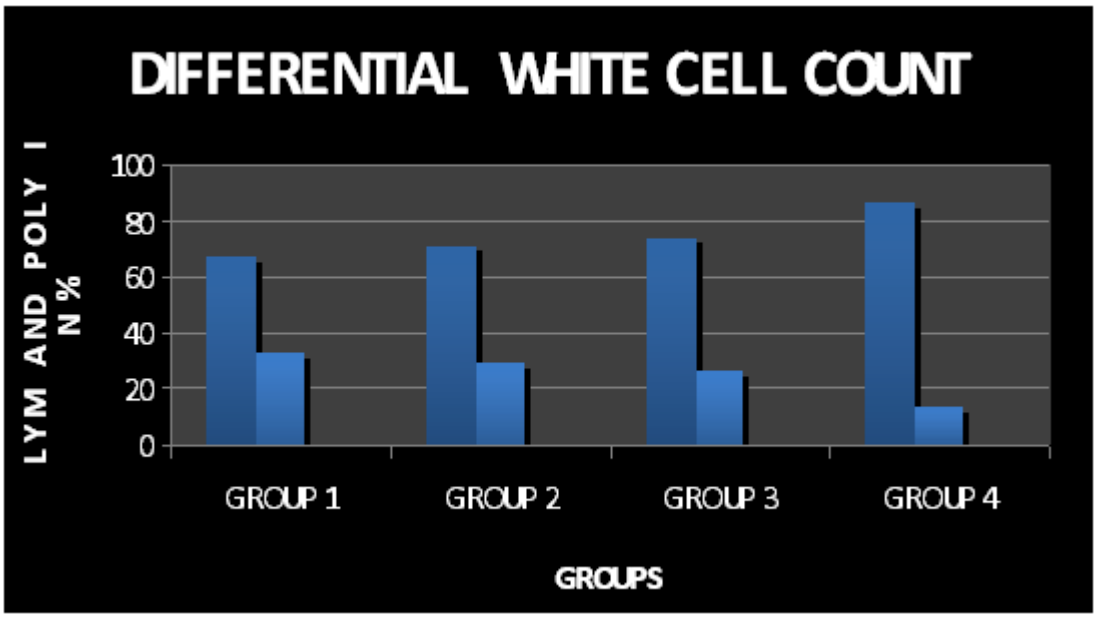

FIGURE 7
CHANGE IN LYMPHOCYTE AND POLYMORPH COUNT IN CONTROL AND TEST ANIMALS DURING Beta vulgaris EXTRACT TREATMENT OF RATS.

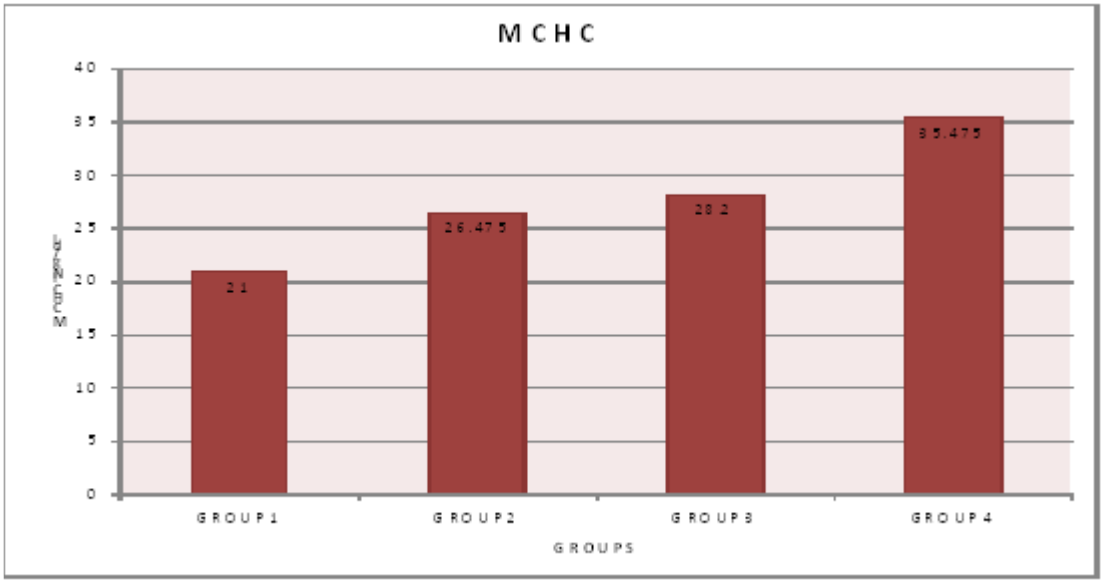

FIGURE 8
CHANGE IN MCHC COUNT IN CONTROL AND TEST ANIMALS DURING Beta vulgaris EXTRACT TREATMENT OF RATS

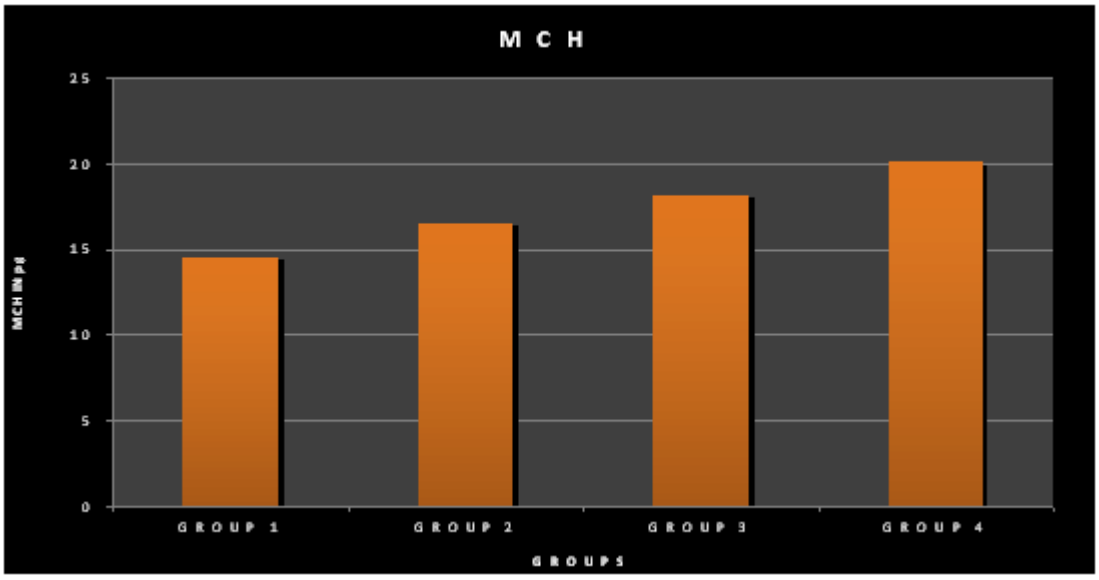

FIGURE 9
CHANGE IN MCH COUNT IN CONTROL AND TEST ANIMALS DURING Beta vulgaris EXTRACT TREATMENT OF RATS

CONCLUSION

It is well known that chemicals, herbs, microorganisms and poisons in tissue response are to some extent species dependent. Consequently, the finding from animal experiment may not be directly interpreted as what might happen in humans, but nevertheless, animals experiment like this provide reliable information of what might happen in humans. In this work, I observed marked increases in packed cell volume, hemoglobin concentration, coupled with raised red blood cell count that could be a result of a direct effect of the extract on the haematopoietic systems. This increase was most pronounced at higher dosage of the extract (400mg/kg). It is therefore reported that *Beta vulgaris* possesses some haematopoietic properties that could possibly be a remedy for anemia.

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