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Short Communication

High Reticulocyte Count with Abnormal Red Blood Cell Morphology in Normal Wistar Rats after Garlic Administration

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Summary: Despite the high acceptability of *Allium sativa* (Garlic) as a remedy for many diseases as earlier stated by many researchers, previous studies have shown that chronic and unregulated consumption of garlic may result to intra vascular haemolytic anaemia in rats. The present study was conducted to examine the effect of crude extract of garlic on microscopic status of red blood cells and some other haematological indices of normal albino rats. The animals were grouped into two; group 1 were normal animals treated with water while group 2 were normal animals administered 150mg/kg body weight of crude extract of garlic on alternate days for three weeks. At the end of three weeks treatment, blood samples obtained from the tail vein of the rats were used for haematological indices and erythrocyte morphology. The values obtained were expressed as Mean± SEM and compared using student t test. The results showed that there was no significant difference in the PCV which was 43.20 ± 0.80% and 45.00 ± 0.36% in both control and experimental groups respectively. However, the RBCs were significantly decreased ($P< 0.05$) from 166.80 ± 3.44 $x10^6$ μL^{-1} in the control group to 87.80 ± 9.34 $x10^6$ μL^{-1} in the treatment group. The percentage reticulocyte counts on the other hand significantly increased from 2.60±4.25% in control group to 11.20± 16.4% in treated group. Fragmented RBCs with a lot of schistocytes with adequate platelets were seen on peripheral blood film of crude garlic treated rats as compared to control. Our results suggested intravascular haemolysis and numerous reticulocytes on blood film confirmed our view on bone marrow response. The presence of schistocytes and acanthocytes may be an indication that the liver is involved in the observed effect.

Keywords*:* Garlic, Red blood cells, Reticulocytes, Rat.

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INTRODUCTION

Garlic (*Allium sativum),* an important bulb vegetable, is used as a spice and flavouring agent of food and medicinal plants throughout the globe. The traditional use of garlic cannot be over emphasised and, in most cases, the plant is consumed in raw form. The effectiveness of garlic in disease amelioration has been scientifically validated (Amagase *et al* 2001; Hussein *et al* 2007) and its anti-glycaemic (Eidi *et al.*, 2006), antioxidant (Jaisuwal and Rizvi, 2011) and antihypertensive (Singh and Singh, 2008) effects have been authenticated through scientific manipulation. Phytochemical study revealed that *Allium sativum* contained organic compounds such as steroids, saponins and flavonoids, it is also characterised by a high content of organo sulphur compound (Singh and Singh, 2008).

Despite high acceptability of *Allium sativa* (Garlic) as a remedy for amelioration of many diseases as earlier stated by many researches (Eidi *et al*; 2006; Hussein *et al*, 2007; Rizvi, 2011), Some Studies have demonstrated that chronic and unregulated consumption of garlic may cause intra vascular haemolytic anaemia in rats (Shashikanth *et al*, 1986; Umar *et al*, 1996) and in dogs (Yamoto and Maeds, 1992). Intra-vascular haemolysis have been attributed to the presence of peroxidisable poly-unsaturated fatty acid and malondialdehyde (MDA) that impair erythrocyte membrane stability (Mansour and Mansour, 2009), this might be the cause of an increased erythrocyte osmotic fragility in rats fed with allium (Salami *et al*, 2012). There are also contrasting views that consumption of aged garlic extracts suppressed deformity and haemolysis rate induced by peroxidation and non-peroxidation, with improved microcirculation (Nasr, 2014).

Some reports have shown that the effectiveness of erythropoiesis can be assessed by quantitative determination of reticulocyte count which are clinical markers of haemolysis (Choi, 2001) while morphology of RBC can also be used as a diagnostic feature of sickle cell anaemia (Olanrewaju, 2002). In view of these reports on garlic consumption, an experiment

was designed to demonstrate the microscopic picture of red blood cell in an attempt to visualise red blood cell morphology and reticulocyte count, since peripheral blood smear is a critical step in the evaluation of any anaemia.

MATERIALS AND METHODS

Plant Materials: Garlic was purchased from the Monday market in Maiduguri, Borno state and authenticated by plant taxonomist in the department of biological sciences, University of Maiduguri, Nigeria.

Animals: Ten (10) adult albino rats weighing between 150g and 250g were obtained from physiology department animal house. The animals were kept in plastic cages at special room in the animal house at room temperature of 30±2 ˚C and less than 30% relative humidity under a 12hours light-dark cycle. They were fed standard pallet food (sanders SPEEC feed Plc. Jos, Nigeria) and had access to water *ad libitum.*

Preparation of Crude Garlic Extract: Five (5) gram of garlic was weighed and then pulverised and mixed with 50ml of distilled water. The preparation served as stock and stored at 4˚C.

Study Design: The rats were grouped into two groups of five (5) rats each. The first group served as control and received 0.2ml of distilled water on alternate days. Group two were treated with crude extract of *Allium sativa* (150mg/kg/day) on alternate days too. The choice of dose was based on previous studies (Umar *et al.,* 1996; Salami *et al.,* 2012). The experiment lasted for 3 weeks; a week corresponds with occurrence of maximal anaemia in rats fed with garlic (Umar *et al*, 1996). At the end of three weeks blood samples were obtained through rat tail after they were anesthetised with ether.

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Determination of Haematological Indices: The blood samples collected were immediately used for haematological indices. The haematocrit or packed cell volume was determined according to the haematocrit method described Alexander and Griffiths (1993). Using a Hawksley microhematocrit reader, the values obtained were expressed in % volume of the blood count occupied by the red blood cell.

Red Blood Cell (RBC) and Reticulocyte Count: The principle was based on counting the number of RBC in a known small volume of accurately diluted blood. Blood was diluted 1:200 with Hayem's solution (an isotonic red blood cells diluting fluid) in a red cell pipette. A small volume of the diluted blood was counted using the improved Neubauer Hemocytometer. The reticulocytes count was carried out using Miller's disc method, and percentage reticulocytes for each rat was calculated using the following formula;

$$\%\ Reticulocyte = \frac{Total\ Reticulocyte}{Total\ RBC}\ X\ 100\ \%$$

Erythrocyte Morphology: A blood film was made by gently touch of fresh drop blood onto one end of a clean grease-free glass slide. Using another glass slide (spreader) that touches the edges of drop blood at an angle of 45^{o}, the spreader was pushed along the slide, drawing the blood behind it, until the whole blood was smeared. The blood smeared was allowed to dry at room temperature and stained using Leishman's for RBC morphology. Reticulocytes are visualized by staining with supravital stain (Brilliant cresyl blue) that precipitate the RNA and organelles. The immature cells (Reticulocytes) appeared microscopically visible as dark-blue clusters and filaments.

Statistical Analyses: The data obtained from the haematological parameters were subjected to statistical analysis using Instat graphpad version 3.05. The values obtained were expressed as Mean± SEM and compared using student t test. P value < 0.05 were considered significant.

RESULTS

Table 1 presents the PCV, RBC and reticulocyte count after 3 weeks of garlic extract administration. There was no significant difference in the PCV between the rats that received garlic (45.00± 0.36%) and control (43.20±0.80%). The mean RBC count of garlic treated rats (87.80±9.34x10^{6} µL^{-1}) was significantly low P<0.001 compared to that of control rats (166.80± 3.44x10^{6} µL^{-1}), while opposite effect was recorded in percentage reticulocyte count with the means 11.20± 16.4% in treated group and 2.60±4.25% recorded in control rats, the difference in percentage reticulocyte count was statistically significant (P<0.005)

Figure 1A shows the blood film of one of the rats fed with garlic showing features of haemolysis i.e fragmented red cells/ schistocytes and red cell agglutination while 1B shows peripheral blood film of red cell from control having normal microscopic blood with adequate white blood cell and platelets.

Figure 2 shows peripheral blood film of another rat fed with garlic showing fragmented red cells/ schistocytes, red cell agglutination, and acanthocytes which infer liver involvement.

Table1. Effect of oral Administration of Garlic Extract on Mean Packed Cell Volume, Red Blood Cell and percentage Reticulocyte count in rats.

	Control	Garlic Treated
RBC (10^{6} µL^{-1})	166.80±3.44	87.80±9.34*
PCV%	43.20±0.80	45.00±0.36
Reticulocytes%	2.60±4.25	11.20±6.43*

*P<0.005

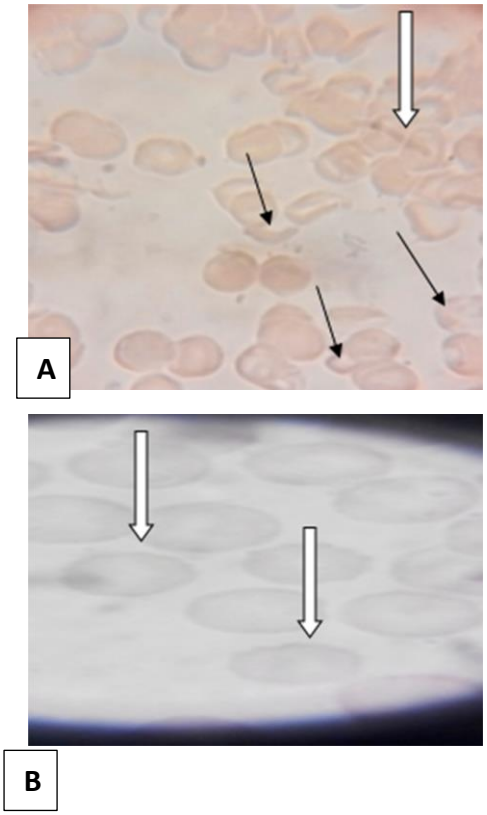

Fig. 1: (A) Shows the blood film of one of the rats fed with garlic showing features of haemolysis i.e fragmented red cells/ schistocytes (slim arrows) and red cell agglutination (bold arrows) MgX40. **(B)** Peripheral blood film of control animals showing normal RBC (bold arrow) X100.

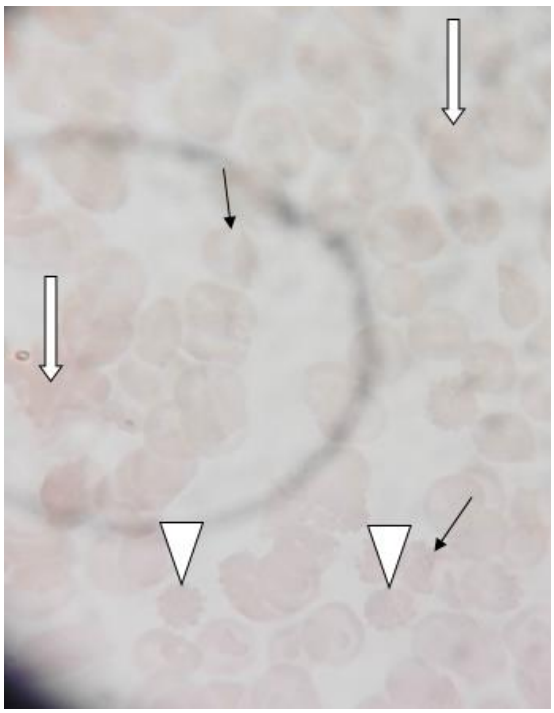

Fig. 2: Shows peripheral blood film of another rat fed with garlic showing fragmented red cells/ schistocyte (slim arrow), red cell agglutination (bold arrow) and acanthocyte (arrow head) MgX40

DISCUSSION

Peripheral blood film was considered in this study because several reports showed that chronic and unregulated intake of garlic causes anaemia (Meleola and Edwin, 1979) of haemolytic type and the degree of heamolysis induced by garlic correlate well with decrease in erythrocyte glutathione (GSH) (Umar *et al*., 1996). This was also confirmed by an increased erythrocyte osmotic fragility of rats fed with garlic (Salami *et al.*, 2012).

In the present study, we observed a remarkable change in morphology of red blood cell both in terms of number and shape. Garlic supplementation showed a feature of intravascular heamolysis (Fragmented cell) and reticulocytosis (the normal response of bone marrow to peripheral loss of red blood cells). The high reticulocyte count recorded in this study agrees with previous study that haemolytic anaemia and other haemopoetic conditions can be monitored by assessing reticulocyte count (Luczynski *et al.*, 2006). Agglutination with a significantly decreased in number of cells both by counting and appearance on peripheral blood film was also seen. This is in support of previous studies that showed peroxides and free radicals caused oxidative damage of membrane; whose stimulation is caused by Garlic (Kashinath, 1990).

Appearance of schistocytes on blood film is suggestive of liver involvement. This is a significant finding because previous studies showed amelioration of garlic on liver damage caused by alcohol consumption (Hussein *et al*., 2007; Shankaran *et al.*, 2010). The lack of unobserved liver damage may probably be due to low quantity of garlic consumed, because study on different concentration of garlic in diet revealed a negative effect at high concentration (Aka *et al*., 2010).

The observed intravascular haemolysis might have resulted into release of haemoglobin and Haem; these two substances have been implicated by some researchers for their involvement in liver cell damage. (Kumar and Bandyopadhayay, 2005). Acute haemolytic anaemia was also reported to induce massive iron accumulation in tissue as one of its characteristic metabolic features (Dhaliwal *et al*, 2004) that can lead to fibrosis of the liver (Brune *et al* 2001).

The data obtained in this study also showed no change in PCV. This is not surprising taking into consideration the high concentration of reticulocytes, a characteristic feature of heamolysis and represent normal response of bone marrow to peripheral blood loss (Gurpreet *et al*,2004).

Thus, our results suggest that oral administration of raw garlic should be monitored especially the liver, if the quantity and duration is mean to be high and long respectively.

Garlic afford a compensatory response by increasing reticulocyte count to counter haemolytic effect. We also observed that garlic attack only old RBCs, this observation is consistent with previous studies that supplementation of high dose of garlic could predispose an animal or human to water loss and metabolic disorder (Aka *et al.*, 2010).

In conclusion, Garlic stimulates erythropoiesis in response to haemolytic effect on RBC membrane as evident by increased reticulocyte circulation and the presence of schistocytes and acanthocytes may be seen as an indication of liver involvement. Thus, our results suggest that oral administration of raw garlic should be monitored, if the quantity and duration is meant to be high and long respectively.

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