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Determination of the Compatibility, UV Resistance of Extracted Plant Pigments in Emulsion Paints and the Effects of Inhalation on Haematological Parameters of Wistar Rats

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Abstract: The present study was designed to determine the effect of extracted plant pigments of Curcuma longa rhizome, Hibiscus sabdariffa flower and inorganic colourants in emulsion paint as it relates to the haematological activities in Wistar rats. The 24-hours acute toxicity test (LD50) of the different mixtures of the colourants in emulsion paint exposed to Wistar rats was determined using Lorke's method. Wistar rats weighing between 120g to 150g were randomized into eight groups (A – H) of five rats each. Different group of rats (groups A to H) were exposed to different mixtures of organic and inorganic colourants in emulsion paint for six months. Group A was exposed to white emulsion paint only, Group B was exposed to 20mls of Curcuma longa paste in white emulsion paint. Group C was exposed to 20mls of Hibiscus sabdariffa pigment in white emulsion paint. Group D was exposed to 20mls (1:1) combination of C. longa and H. sabdariffa. Group E was exposed to 20mls of C. longa paste and inorganic yellow oxide paste in white emulsion paint, Group F was exposed to 20mls (1:1) combination of H. sabdariffa paste and inorganic red oxide paste in white emulsion paint. Group G was exposed to 20mls (1:1) combination of yellow oxide paste and red oxide paste in white emulsion paint. Group H was the Normal Control which was not exposed to paint. All animals were allowed free access to commercial rat mash and distilled water throughout the six months of exposure. The results revealed that the 24-hour acute toxicity test did not record any mortality. The Curcuma longa paste and Hibiscus sabdariffa paste were completely miscible and compatible with other components of the paint although they degraded or faded in colour faster on exterior application than interior application. The fading of the colours exposed externally compared to the ones exposed internally can be attributed to the effect of sunlight, rain and intense temperature from the ultra-violet rays. the exposure of the rats to the different paint samples reveal a non- significant increase or decrease in the haemoglobin concentrations compared to normal control group H from 2nd to 6th month apart from group D which showed a significant decrease ($p<0.05$) in haemoglobin concentration within the first month of the experiment compared to normal control. Group C and D also showed a significant decrease ($p<0.05$) in the packed cell volume concentration at 5th month and 1st month of exposure respectively compared to the normal control (group H). There was a significant decrease ($p<0.05$) in the red blood cells of group D compared to the control group H at the first month. Also, a significant increase ($p<0.05$) in the concentration of the white blood cell was noticed on first month of exposure compared to the control group H. However, it was also interesting to note the compatibility of the plant extracts with conventional paint components but the alterations in the red blood cells and white blood cells could be attributed to the level of toxicity of the different paint samples and prolonged inhalation of the paint fumes by the wistar rats.

Key points: Curcuma longa, rhizome, Hibiscus sabdariffa, haematological parameters, toxicity.

INTRODUCTION

Paints are homogenous mixtures or solutions, which when applied on a substrate, binds and dry to form a smooth continuous hard film. It has the ability to adhere to a desired surface (substrate) on which it is applied either by hand brush, paint roller or spraying gun. The conversion process can be physical (e.g loss of solvent) and or chemical (i.e low or high temperature polymerization and is variously called drying, curing or cross linking). Paints consist of three major components which include solvents, pigments and binders. Other specialty materials are grouped under additives. (Karakas *et al.,* 2015).

Paints are generally categorized under two groups, namely, water-based and oil-based coating systems. The water-based paints have water as their major solvent or lubricant while oil-based paint may have hydrocarbon, aromatic or ester etc. as solvent (Shah *et al.,* 2022). Water-based and oil-based paints are predominantly used both for interior and exterior applications for decorative, protective and industrial purposes. Emulsion paints are simple, single component water-based paints produced ready for use. The emulsion paints are manufactured for decoration purpose and are often present in a wide range of colours. Colourants either in paste or pigment form are usually incorporated during colour matching or tinting process or during pigment grinding contingent on the dispersion stage of the production process. Meanwhile, the pigments or colourants are the sole determinant of the final colour of the paint. Besides, they are solely responsible for the opacity and light fastener of the paint after application (Fernandez and Depablo, 2022).

Paints workers are exposed to various grades of harmful chemicals present in paint products such as solvents (aromatic hydrocarbons e.g benzene, toluene and xylene (Roma-Torres *et al.,* 2006). Also, paints contain pigment such as lead, cadmium, arsenic and chromium (Awodele *et al.,* 2014). Besides, titanium dioxide and silver are nano-particles used as paint additives (Smulders *et al.,* 2014). All these constituents were reported by many studies to have adverse effects on neurobehavioural, blood, kidney, liver, cardiac, respiratory functions, spleen and many body systems (Chen *et al.,* 2001; Ridgway *et al.,* 2013; Agin *et al.,* 2016). Toxic effects of these materials on the DNA and blood can contribute to their carcinogenicity (Scelo *et al.,* 2009).

Generally, inorganic pigments exhibit better stability against sunlight in paints compared to the organic equivalents, but the shades and hues of the former are normally dull compared to the sharper and brighter shade and the of the latter. Therefore, to obtain some desired colours, for decorative purposes, it becomes imperative that there must be a blend of both organic and inorganic pigments. Many fruits and vegetables naturally have pigments.

They have severally compounds with particular characteristics: the main four being chlorophylls (green), carotenoids (yellow, orange red), betalains (orange) and anthocyanins (red-blue, purple) (Rodrignez and Amaya, 2016). The principal natural pigments are soluble in water or liquids and organic solvents significantly different in both structure and metabolic pathways (Zhang *et al.,* 2014). Commercially, anthcyanins, betalains and carotenoids have extensive use for red, orange and yellow shades respectively (Da-costa *et al.,* 2014).

Curcuma longa rhizome and *Hibiscus sabdariffa* are now common plants used to enhance the properties of paint products thereby minimizing the toxic effect of the raw materials used to produce indoor and outdoor paints. With the hyperinflation in the country, the prices or virtually all the raw materials, including the pigments and colourants which are basically imported are very costly and therefore make the final cost paints to be beyond the reach of the discerning end users. Therefore, this study was aimed at investigating the colouring potential of the plant extracts, *Curcuma longa* rhizome and *Hibiscus sabdariffa,* their compatibility and ultra violet rays resistance in water-based paints as well as the effect of prolonged inhalation (on exposure) on the red blood cells, white blood cell and their differentials in wistar rats.

MATERIALS AND METHODS

Collection of the plant samples

Turmeric rhizome (*Curcuma longa*) and Zobo flowers (*Hibiscus sabdariffa*) were purchased from street market at Etuk street, Uyo, Akwa Ibom State, Nigeria. The samples were validated by a Taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State.

One gallon of white clover emulsion paint was purchased from paint depot at Abak Road, Uyo Local Government Area of Akwa Ibom State, Nigeria. All the samples were conveyed to biochemistry laboratory at Nnamdi Azikiwe University, Awka, Anambra State were they were used for preparation of different coloured paint samples and use in the study.

PREPARATION OF THE PLANT COLOURANTS

The turmeric rhizomes were washed, peeled and rinsed with distilled water to remove all contaminants. The samples were chopped into tiny pieces and dried at room temperature for two weeks. The zobo flowers (*Hibiscus sabdariffa*) were washed with distilled water and air dried at room temperature for two weeks. The dried *Curcuma longa* and *Hisbiscus sabdariffa* were ground into powder using corona manual grinding machine. Exactly one (1kg) each of the dried pulverized *Curcuma longa* and *Hibiscus sabdariffa* were separately soaked in 5l of 70 % ethanol each. The mixture was stirred at 2 hours interval to ensure complete extraction of the pigment. After 2-hours, the mixtures were separately sieved using muslin cloth and thereafter filtered with what mann no 1 filter paper. The filtrate was concentrated into a paste or slurry at 50°C using water bath. The weights of both pigments were recorded. The extract was stoppered in a universal container and stored in the refrigerator at 4°C for use in colour matching or tinting or paints.

EXPERIMENTAL DESIGN, GROUPING AND TREATMENT OF THE ANIMALS

Fourty (40) male wistar rats weighing 120g – 150g were purchased from the disease free stock in the Chris Experimental Animal Farm and Research Laboratory, Awka and were randomized into eight groups of five (5) rats each.

Group A: White emulsion paint only.

Group B: 20mls of *C. longa* in white emulsion paint.

Group C: 20mls of *H. sabdariffa* in white emulsion paint.

Group D: 20mls combination of *C. longa* and *H. sabdariffa* in white emulsion paint.

Group E: 20mls of *C. longa* and inorganic yellow oxide paste in white emulsion paint.

Group F: 20mls of *H. sabdariffa* and inorganic red oxide paste in white emulsion paint.

Group G: 20mls of yellow oxide paste and red oxide paste in white emulsion paint.

Group H: Normal control

The combination in group D, E, F, G were all 1:1 ratio. The twenty milliliters of each paint samples was put inside a small metal tin and hung inside the cage at the level the animals could not ingest but rather inhaled the paint fumes. Besides, the old samples of paints were replaced with fresh samples on weekly bases. All animals were allowed free access to commercial rat mash and water throughout the six months of experiment. Good ventilation and hygiene was strictly maintained by constant cleaning and removal of faeces and spilt feeds from the cages daily.

COLLECTION OF BLOOD SAMPLE

After every one month interval, blood samples were collected from the eyes of the experimental animals ocularly and used for haematological analysis. At the end of six months exposure of the animals to paint fumes, the animals were fasted overnight, anaesthetized under chloroform vapour, sacrificed by dissecting medioventrically and blood samples were collected via cardiac puncture by means of syringe and needles into a well labeled anticoagulant (EDTA) sample bottles and gently

shaken. The blood sample was used for assay of red blood cells (RBCs), and white blood cells (WBCs), Heamoglobin (Hb), Packed Cell Volume (PCV), Platelets, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Procakitonin (PCT), Neutrophil, Lymphocyte, Monocytes, Eosinophil and Basophils.

LABORATORY ANALYSIS

Full blood count was determined using automated haemotology analyzer (Mindray-BC 28000) a three auto analyzer to test 19 parameters per sample including RBC count, HCT, MCH, MPV, LYM, RDWCV and PLT count. Standardization and calibration of the instrument were done according to the manufacturer's instruction.

PROCEDURE

Each blood sample was well mixed then approximately 20ml was aspirated by allowing the analyzer's sampling into the blood sample and dressing the start button. Results of the analysis were displayed after 30 seconds which the analyzer generated a paper copy of the results on thermal printing paper.

STATISTICAL ANALYSIS

Data obtained from the experiments were analyzed using the statistical package for social sciences (SPSS) software for windows version 21. Statistical analysis of the results obtained were carried out using one-way analysis of variance (ANOVA) and POS-HOC tests to determine if significant difference exists between the mean of the lest and control group. The limit of significance was set at $p<0.05$.

RESULTS

Results of Haematological Analysis

The haematological parameters analysed at monthly interval for a period of six months included haemoglobin (Figure 1), packed cell volume (Figure 2), red blood cells (Figure 3), platelets (Figure 4), white blood cells (Figure 5), mean corpuscular volume (Figure 6), mean corpuscular haemoglobin (Figure 7), mean corpuscular haemoglobin concentration (Figure 8), procalcitonin (Figure 9), neutrophils (Figure 10), lymphocytes (Figure 11), monocytes (Figure 12), eosinophils (Figure 13), and basophils (Figure 14). The bar-charts indicated in each figure represent the haematological parameters for a period of six months analysed at monthly interval.

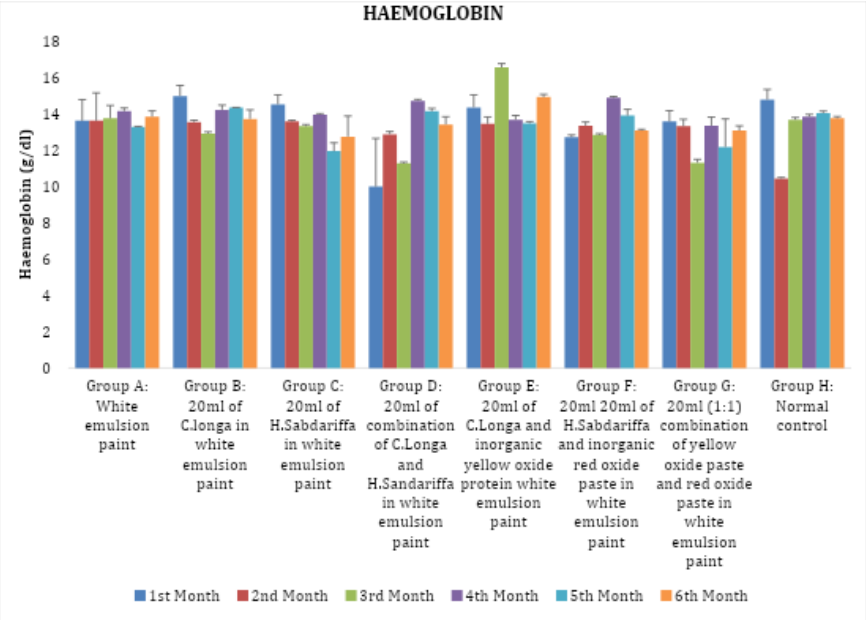

Figure 1: Effect of *C. longa* and *H. sabdariffa* mixed with paint on haemoglobin of Wistar rats expressed.

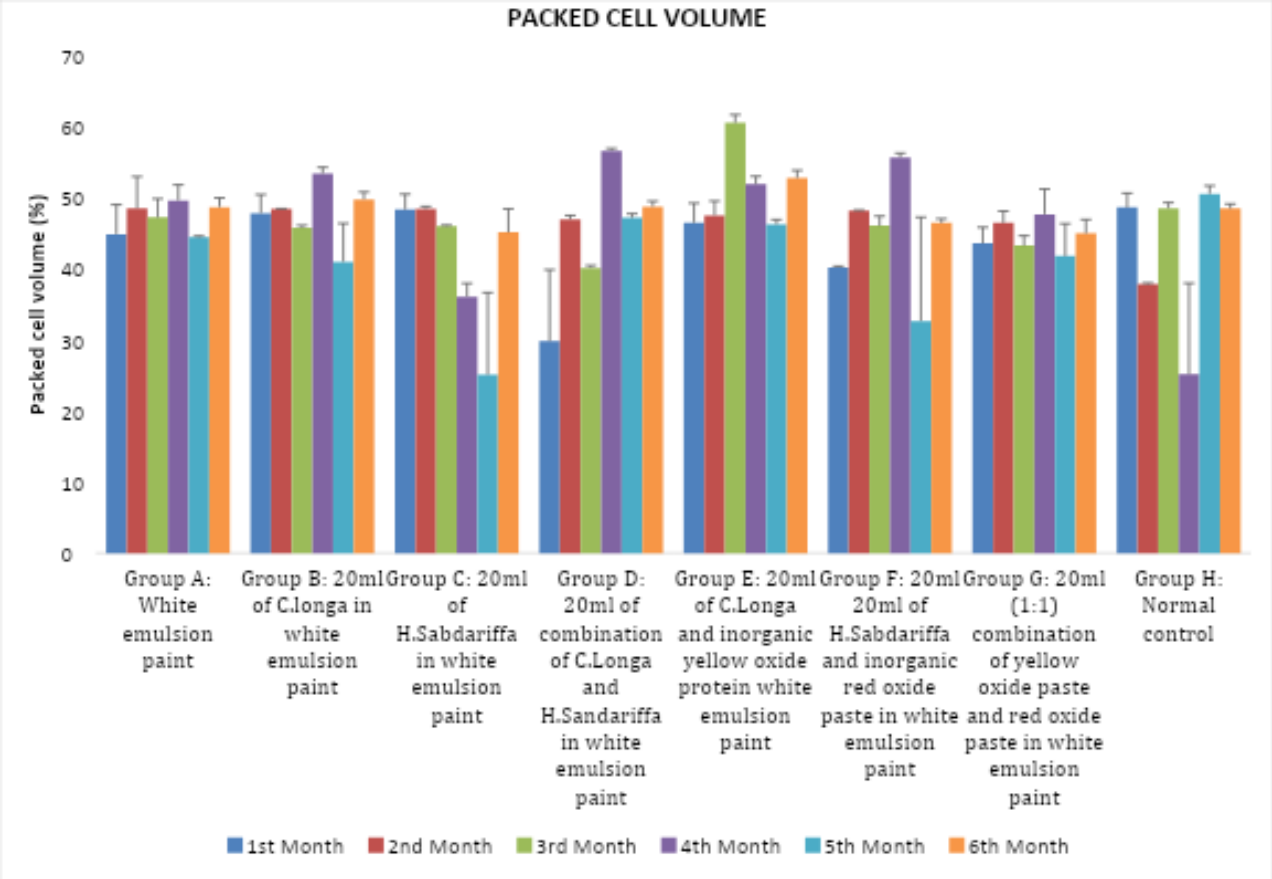

Figure 2: Effect of *C. longa* and *H. sabdariffa* mixed with paint on packed cell volume of Wistar rats.

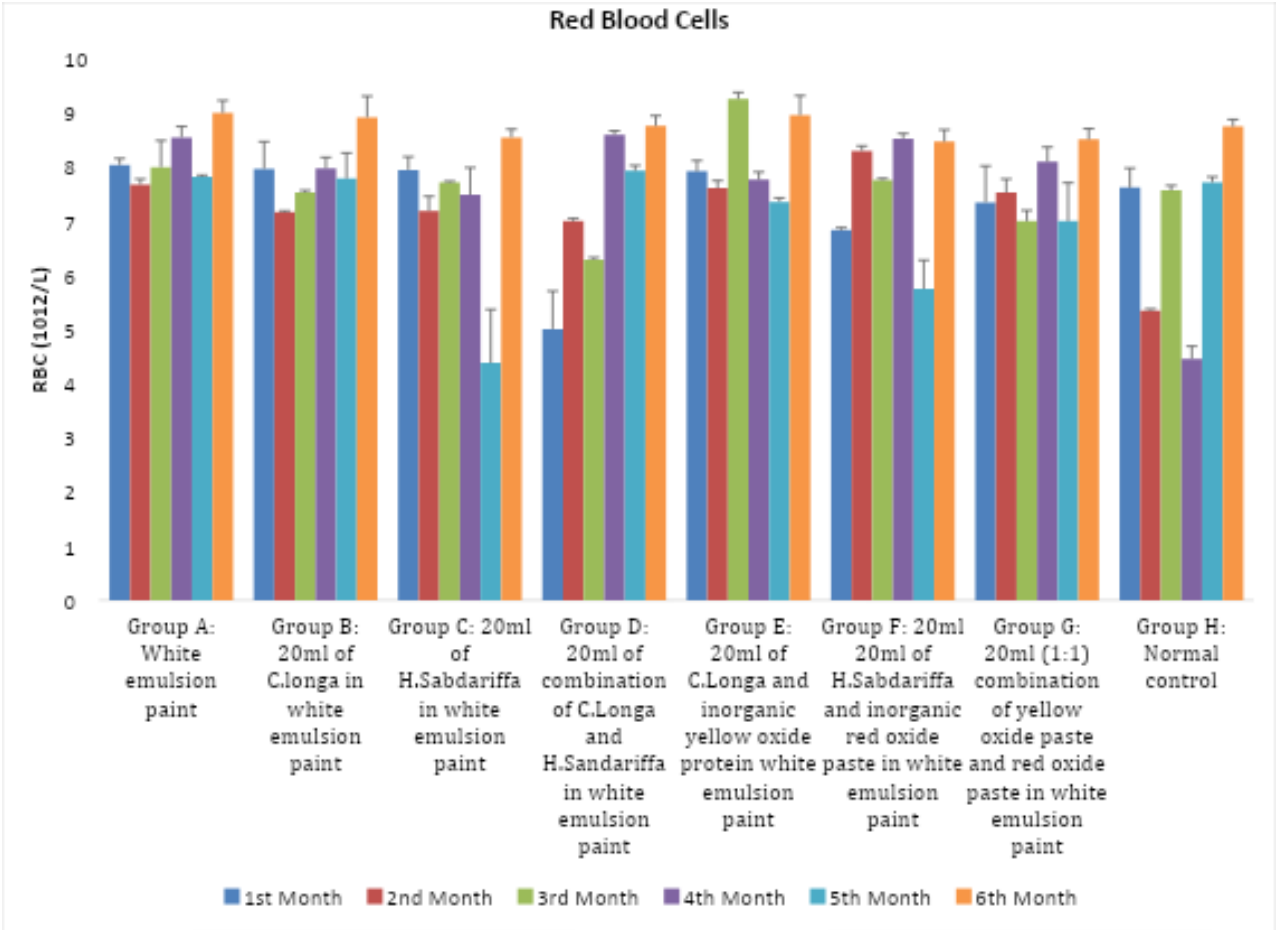

Figure 3: Effect of *C. longa* and *H. sabdariffa* mixed with paint on red blood cell of Wistar rats.

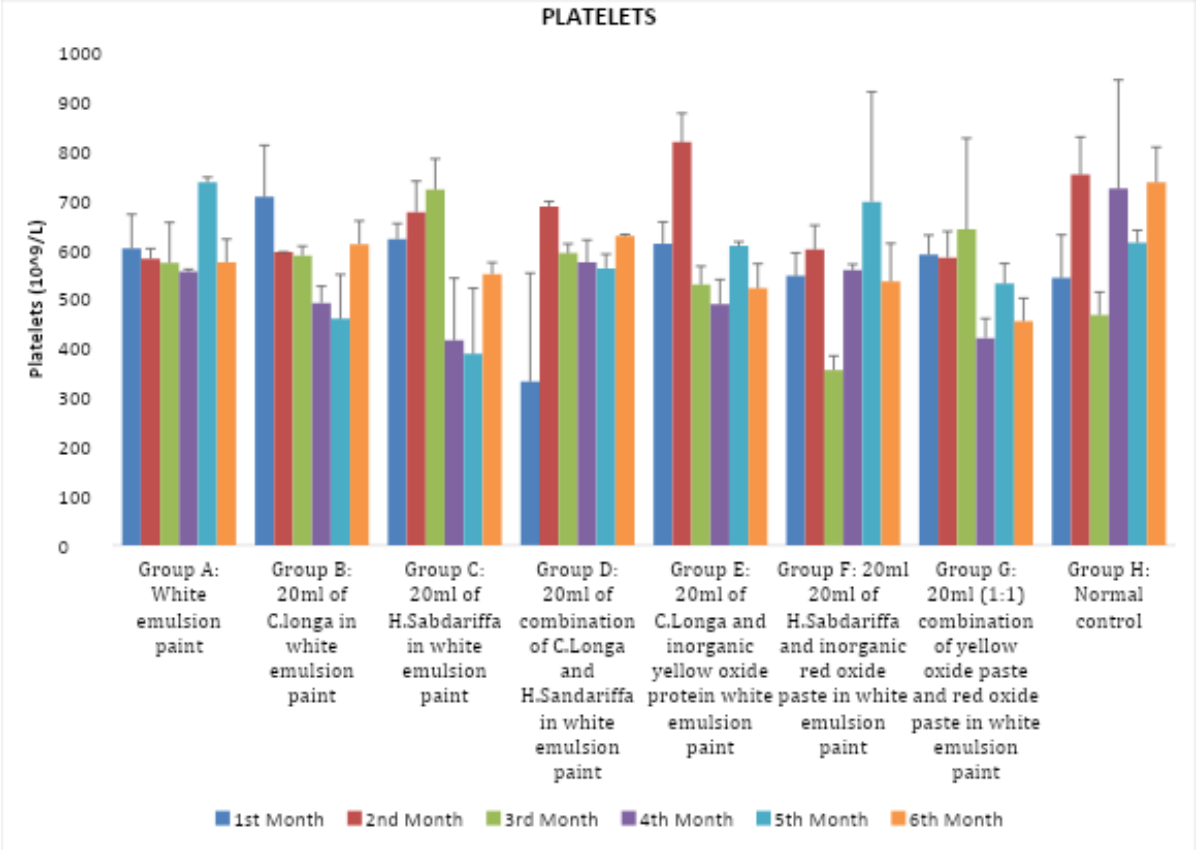

Figure 4: Effect of *C. longa* and *H. sabdariffa* mixed with paint on platelets of Wistar rats.

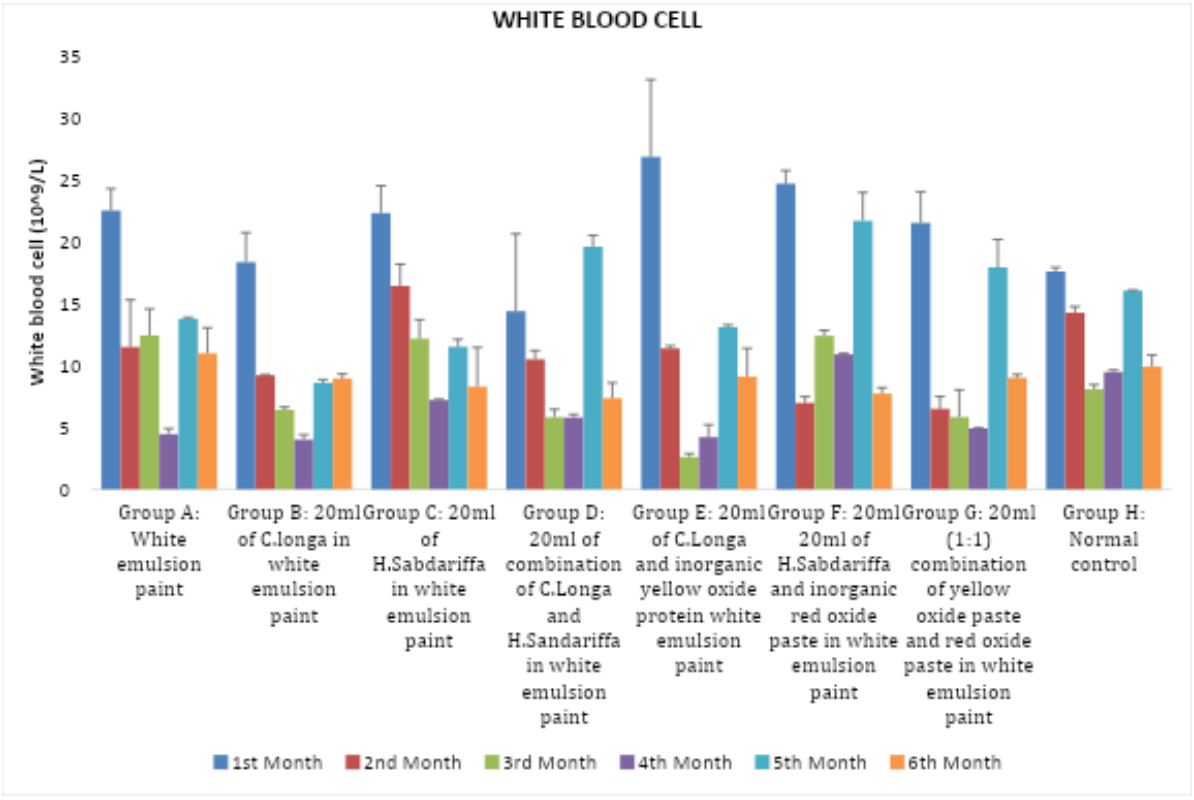

Figure 5: Effect of *C. longa* and *H. sabdariffa* mixed with paint on white blood cell of Wistar rats.

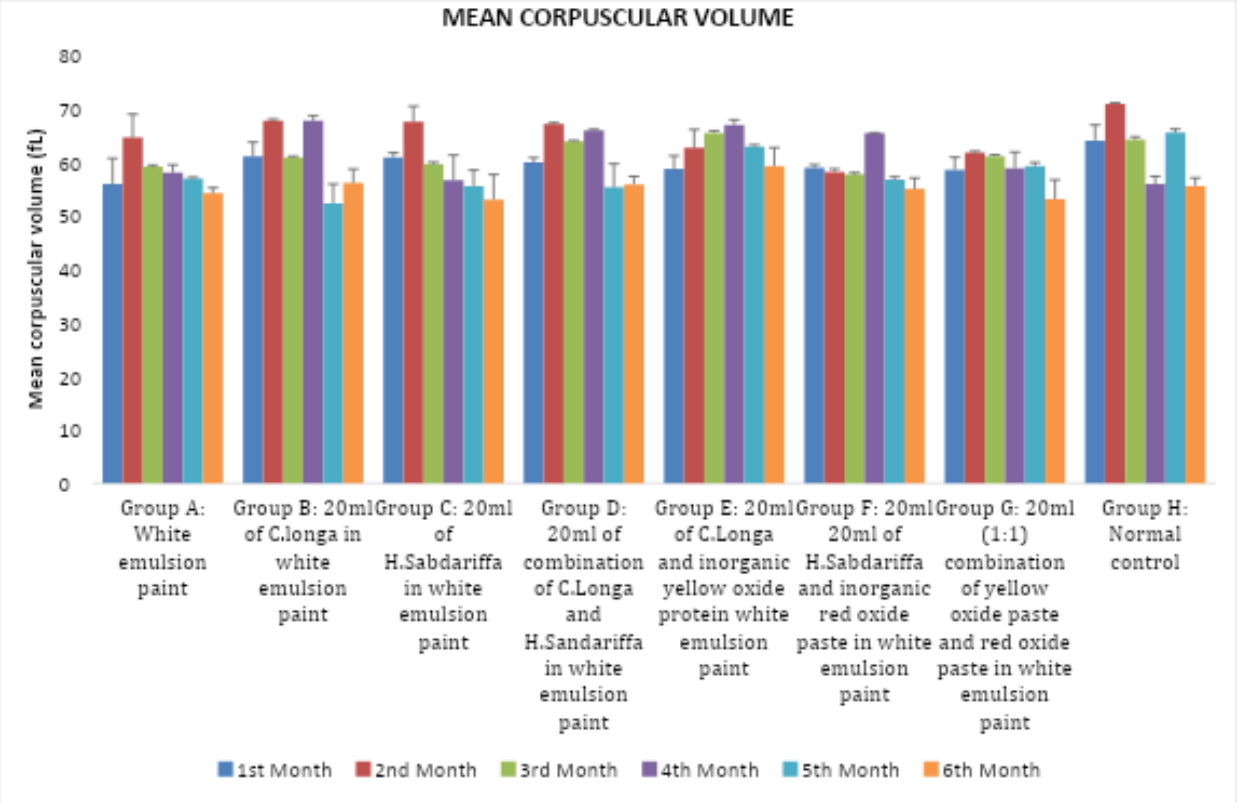

Figure 6: Effect of *C. longa* and *H. sabdariffa* mixed with paint on mean corpuscular volume of wistar rats.

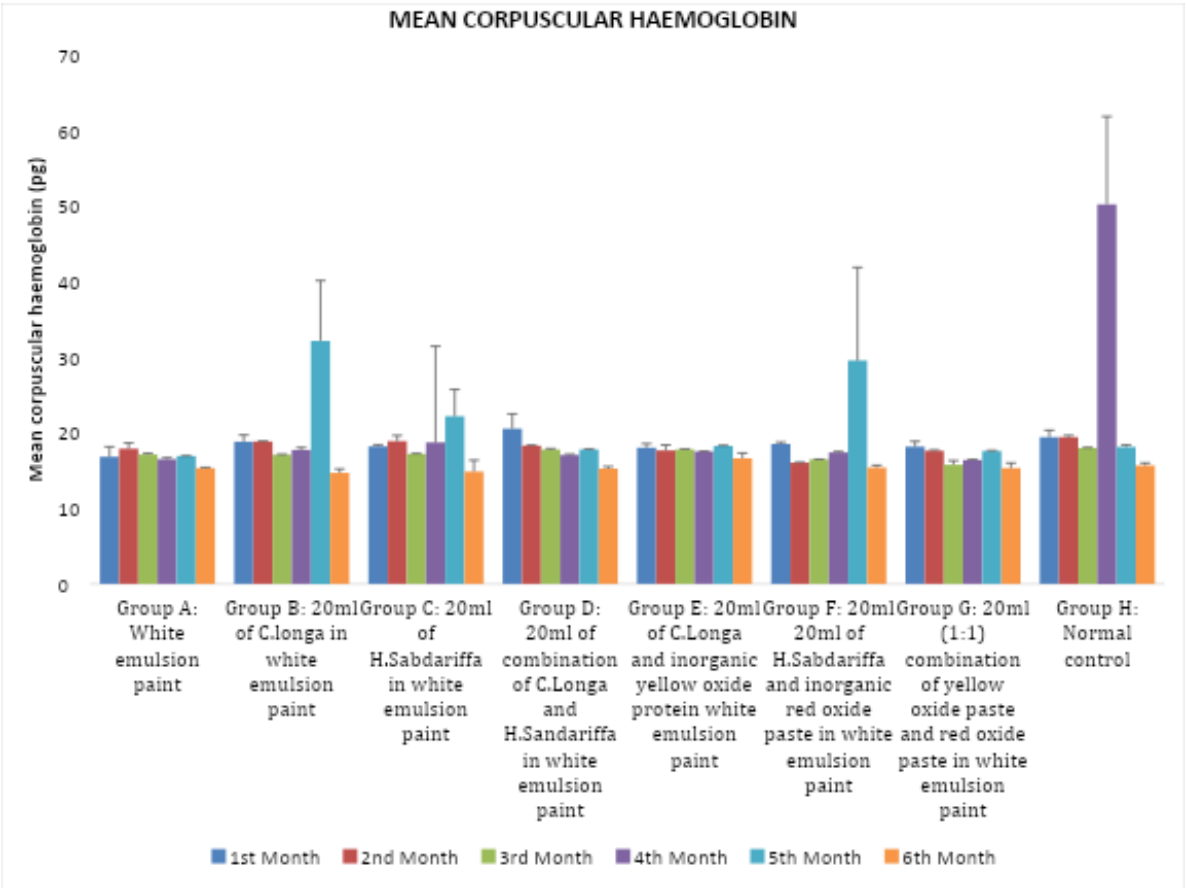

Figure 7: Effect of *C. longa* and *H. sabdariffa* mixed with paint on mean corpuscular haemoglobin of Wistar rats.

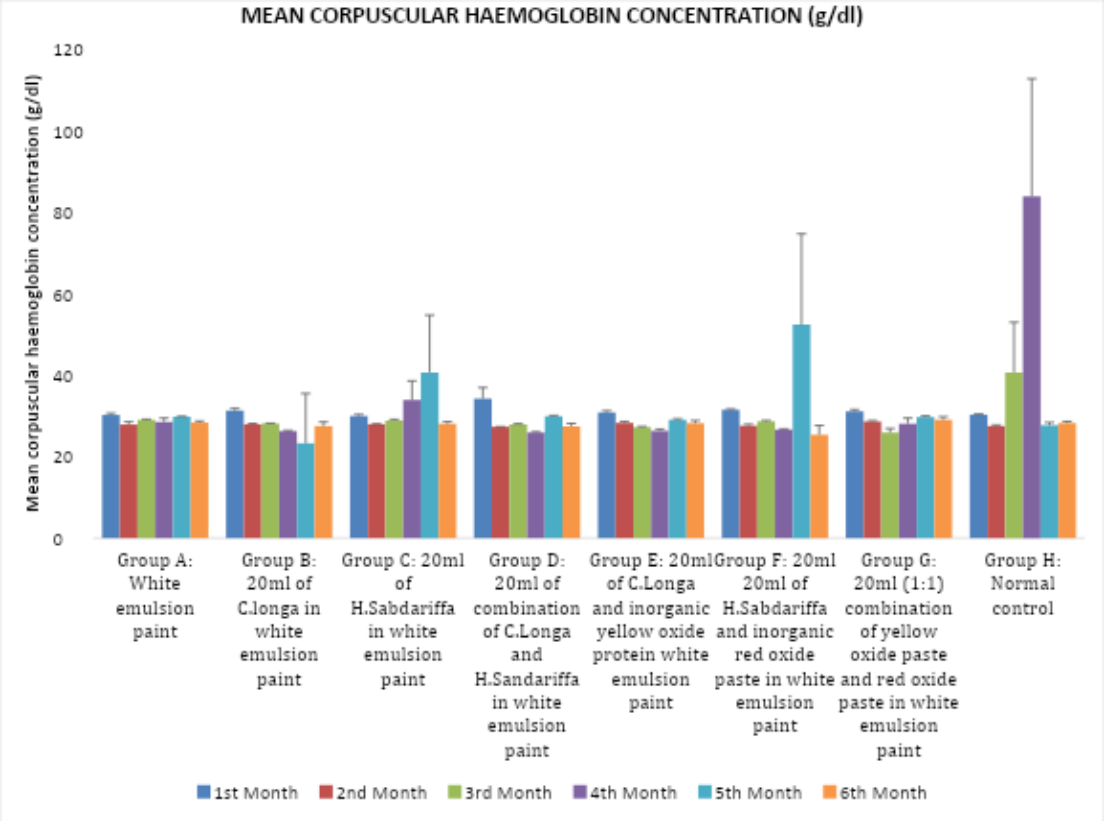

Figure 8: Effect of *C. longa* and *H. sabdariffa* mixed with paint on mean corpuscular haemoglobin concentration of Wistar rats.

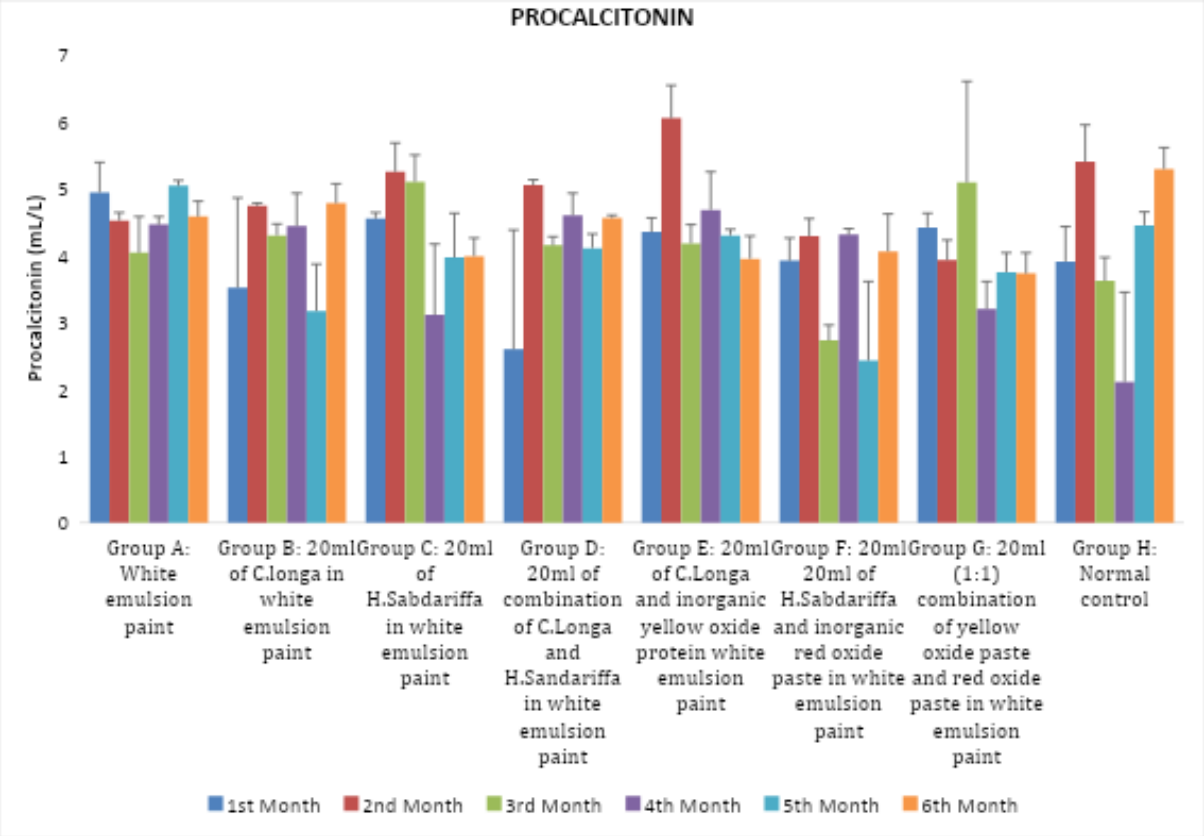

Figure 9: Effect of *C. longa* and *H. sabdariffa* mixed with paint on procalcitonin of Wistar rats.

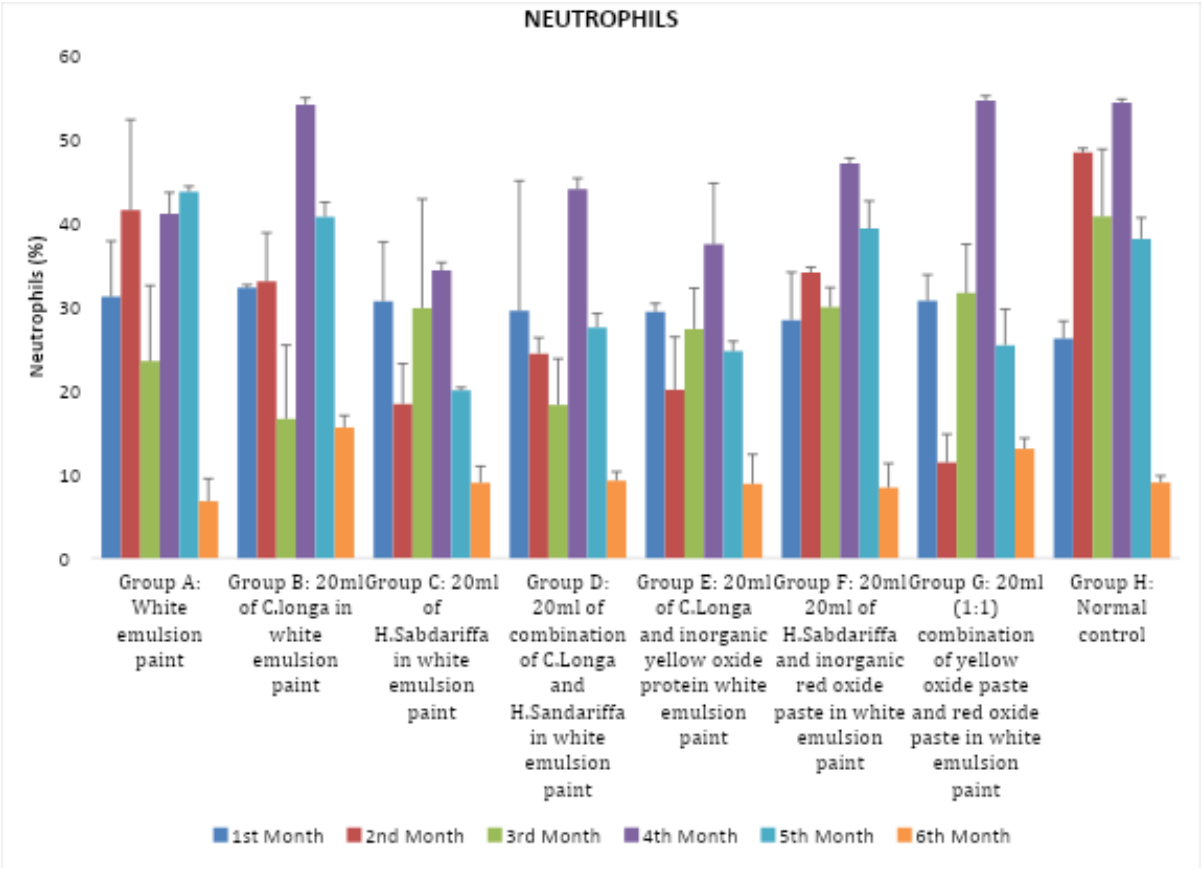

Figure 10: Effect of *C. longa* and *H. sabdariffa* mixed with paint on neutrophils of Wistar rats.

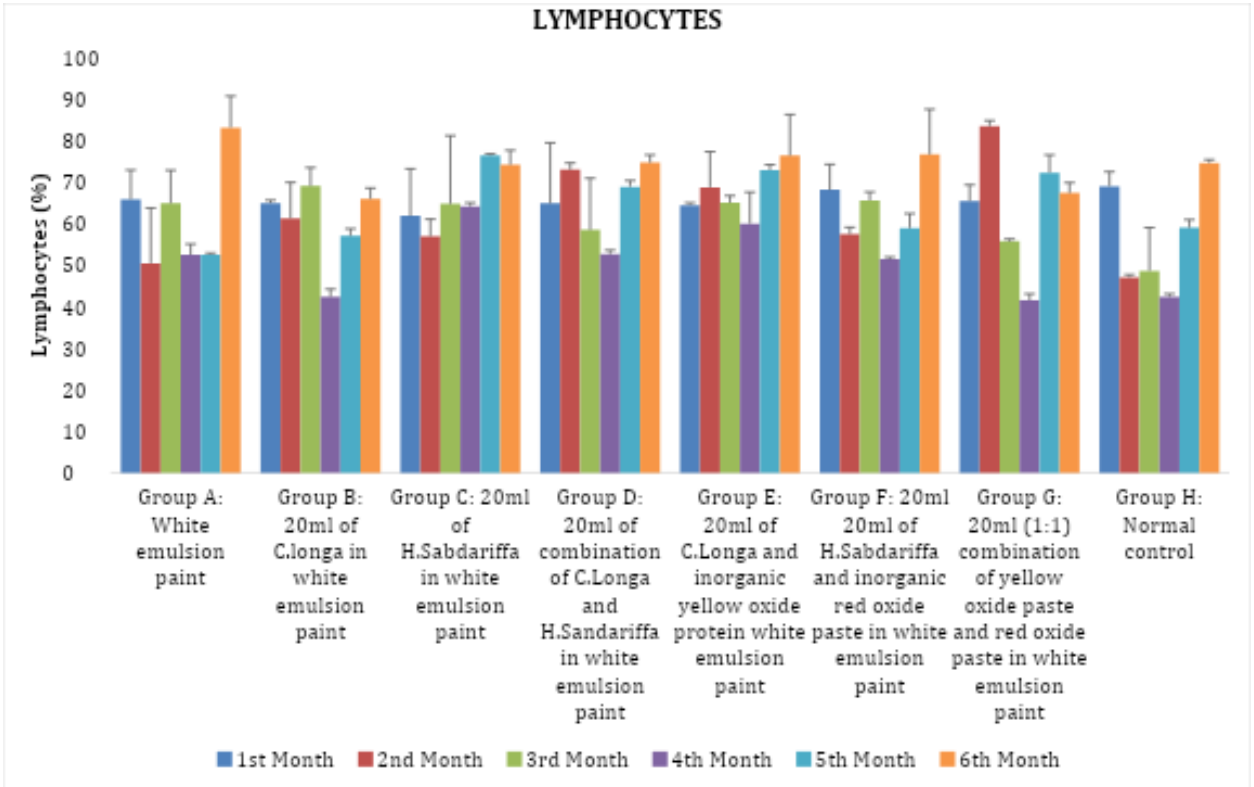

Figure 11: Effect of *C. longa* and *H. sabdariffa* mixed with paint on lymphocytes of Wistar rats.

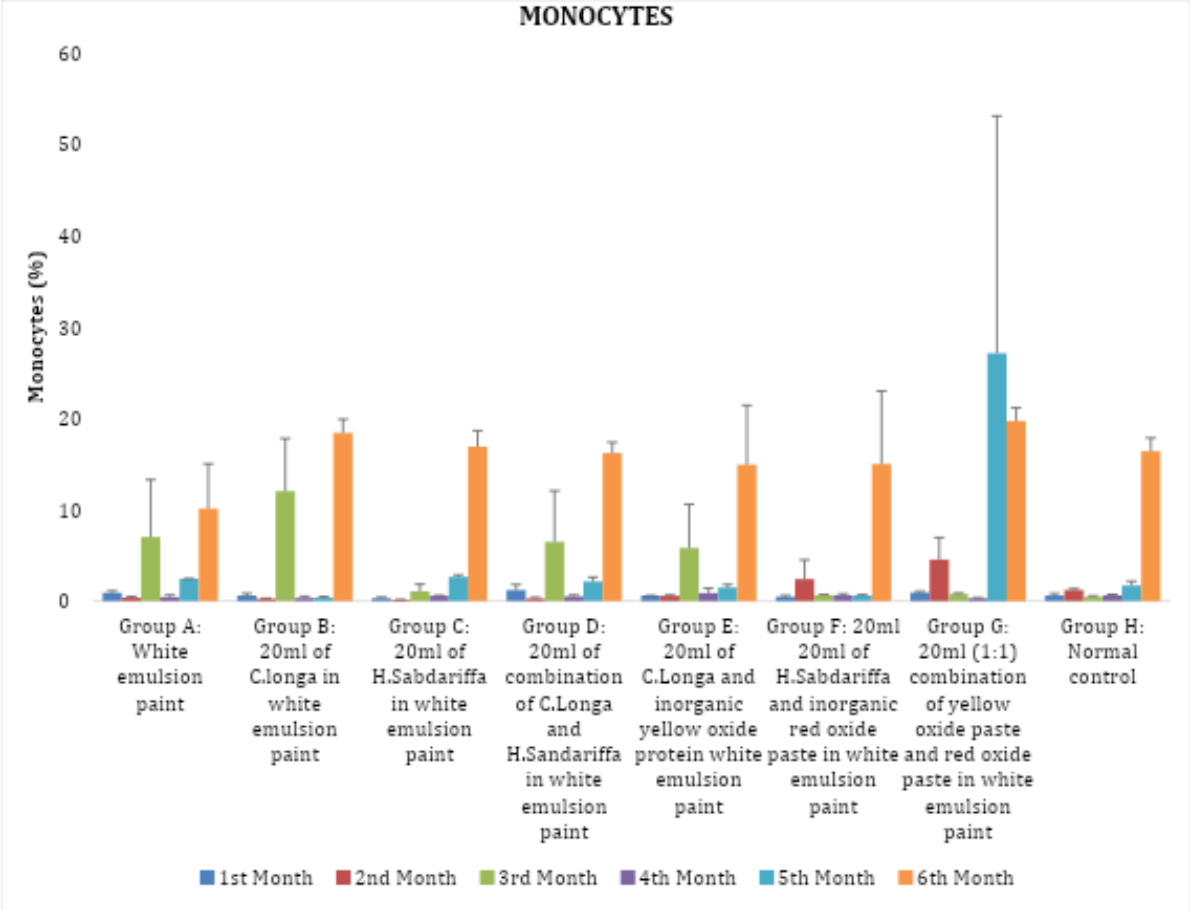

Figure 12: Effect of *C. longa* and *H. sabdariffa* mixed with paint on monocytes of Wistar rats.

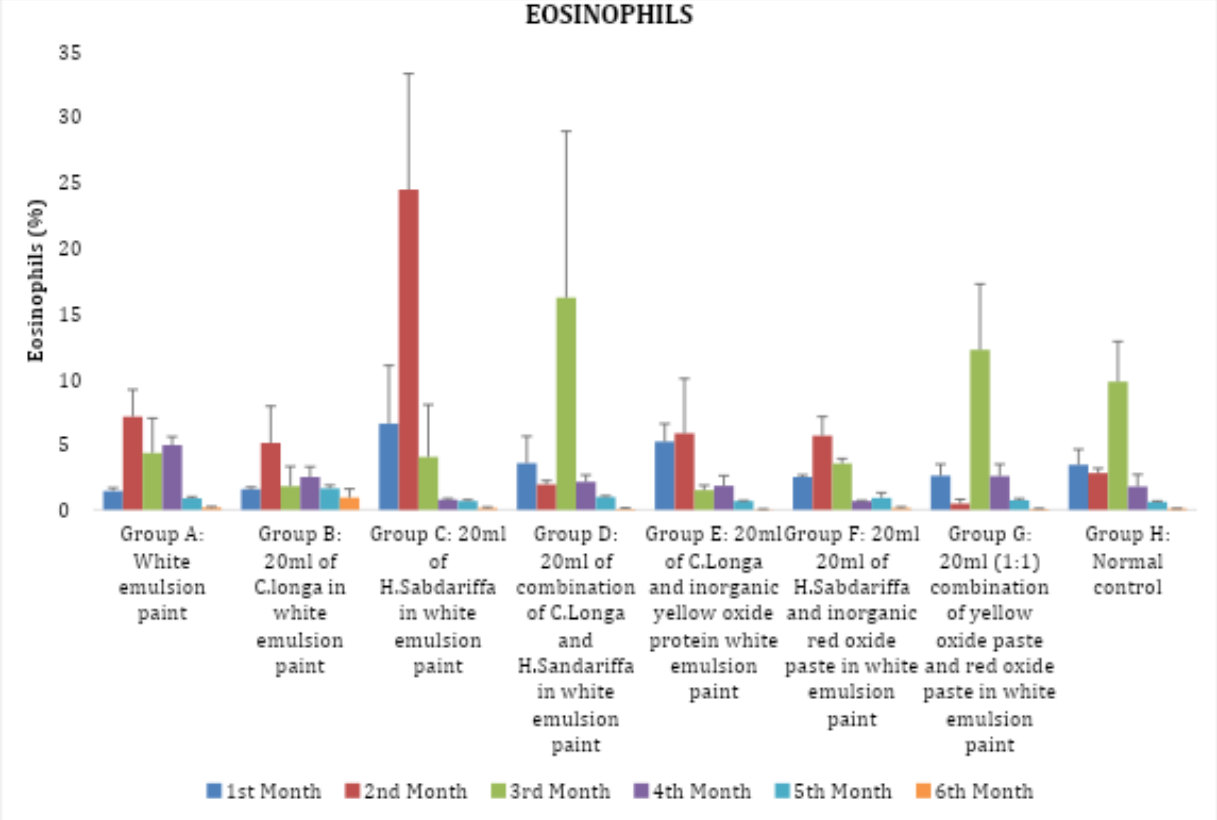

Figure 13: Effect of *C. longa* and *H. sabdariffa* mixed with paint on eosinophils of Wistar rats

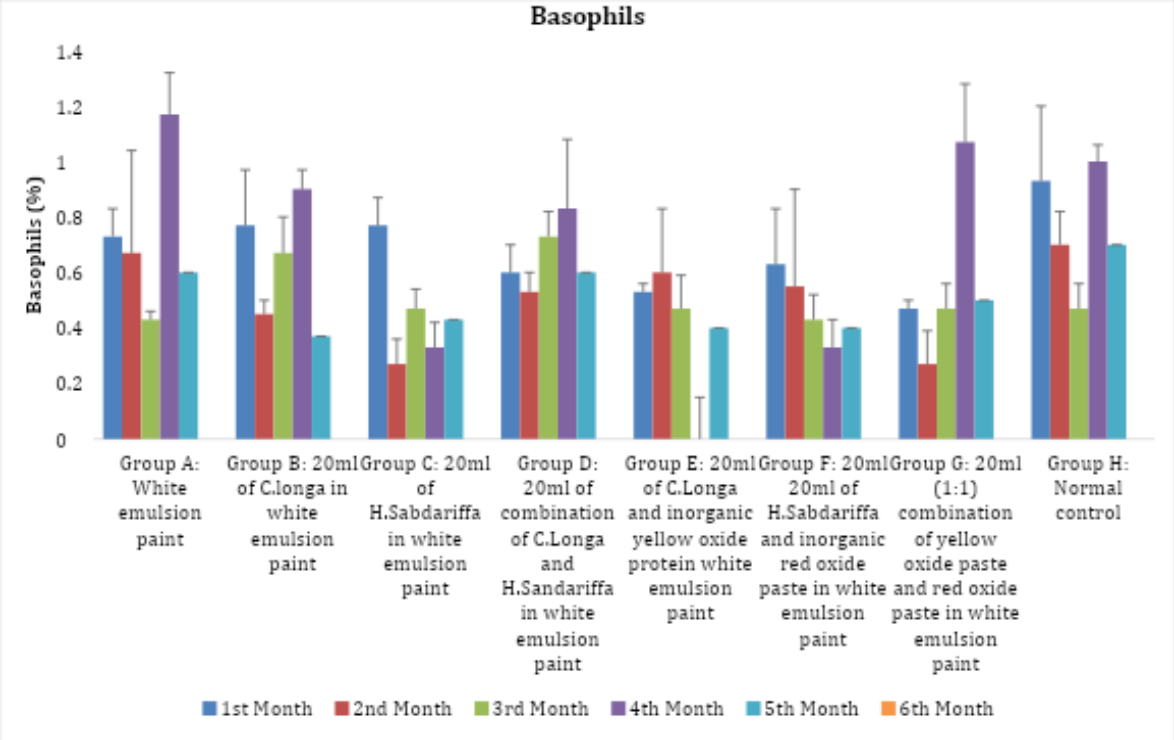

Figure 14: Effect of *C. longa* and *H. sabdariffa* mixed with paint on basophils of Wistar rats.

DISCUSSION

Haematological indices in living organisms are useful parameters for investigating the physiological state of the organism (Khan and Zafar, 2005). They serve as effective diagnostic tools for blood related disorders which include anaemia, thrombocytepenia and immune-deficiency infections.

Meanwhile, the prolonged exposure of the experimental animals to the fumes that exuded from the paint samples showed significant alterations in the red blood cells, white blood cells and some of their differentials in all the groups at the 2nd and 4th month when compared to the control group.

Haemoglobin level revealed a non-significant difference ($p>0.05$) in the groups exposed to different paint preparations except Group D that was exposed to 20 mls (1:1) combination of *C. longa* and *H. sabdariffa* in white emulsion paint which showed a significant decrease ($p<0.05$) within the first month of experiment when compared to the normal control. Haemoglobin is the iron-containing oxygen transport metalloprotein in the red blood cells of almost all vertebrates as well as the tissues of some invertebrates. It transports oxygen from the lungs or gills to the tissue and in turn transports carbon (iv) oxide from the tissues to the lungs for exhalation. This process enhances aerobic respirations which provide energy to power the physiological activities of the organisms in a process termed metabolism. Low concentration of haemoglobin than the normal range of 11 – 13% is an indication of anemia.

Group D also showed a significant decrease ($p<0.05$) in the packed cell volume (PCV) concentration within the first month of exposure compared to the control group H. other names for PVC include, hematocrite, volume of packed red cells (VPRC) or erythrocyte volume fraction (EVF) the normal level of PCV for men is 40.7% to 50.3% and 36.1% to 44.3% for women (Medline Plus, 2019) Higher concentration of PCV (Polycythemia) than normal can indicate a blood disorder, dehydration or other medical conditions (Blix and Hedlin, 2001). Whereas, an abnormally low PCV may suggest anemia which is a decrease in the total amount of red blood cells.

Group D also showed a significant decrease ($p<0.05$) in the red blood cells concentration during the first month than the control group H. Red blood cells (RBC) contains haemoglobin which enhance the redish colouration of the blood and plays prominent role in transportation of oxygen to the cellular tissues of the organism and propels the release of Adenosine triphosphate contingent on

shear stress in constricted vessels which causes the vessel walls to relax and dilate so as to promote normal blood flow (Wan *et al.,* 2008; Diesen *et al.,* 2008).

There was a significant decrease ($p<0.05$) in the platelet concentration of group D compared to control group in the first month of the experiment. Also, the result revealed a significant reduction ($p<0.05$) in the platelet level of Group G compared to the control group in the 4th, 5th and 6th months of exposure. Platelets play an important role in the maintenance of normal homeostasis and effective wound healing. Again, as the experiment progressed to the third and sixth months, the white blood cell concentration showed a significant reduction ($p<0.05$) when compared to the normal control group H. The reduction in the level of white blood cell could be attributed to increased infection which the white blood cells help to fight as it often protects the body against infections disease and foreign invaders. Normal level of WBC is 4 x 109/C or 1.1 x 10a/L and immunity depends on WBC. MCV recorded significant increase on the first month compared to normal control. MCHC showed significant increase ($p<0.05$) in groups A, B, C, D, E, F and G compared to normal group H. On the fourth month of the experiment as well as the fifth month for group F. Meanwhile, normal MCHC in adults is 31 – 35g/dL.

MCH and MCHC ensures the functionality and membrane integrity on the red blood cells.

The procalcitonin level showed a significant decrease ($p<0.05$) in group D on the first month compared to the normal control. While a significant decrease was also noticed in group F on the 5th month compared to the normal control.

The neutrophil concentration in groups B, D, E and G showed a significant decrease ($p<0.05$) on the 2nd month. However, there was a significant reduction in the concentration of neutrophil of groups A – G including the normal control on the 6th month in contrast to the first to fifth month.

Meanwhile, the normal concentration of MCH, MCHC and neutrophil are 18.37 – 36.98pg (Delwatt *et al.,* 2001) 31 – 35g/dL and 40 – 75% (adults), 20 – 45% (children) respectively the lymphocytes also showed a significant reduction in group D and G compared to normal group in the 2nd month. Also, a significant increase ($p<0.05$) was noticed in the lymphocytes of group C in the 5th month compared to the normal standard monocytes, eosinophils and basophils concentrations in group A, B, C, D, E and F compared to normal control H in the 2nd month of the experiment.

However, there was no significant difference in the concentration of basophils on any month during the experiment.

Lymphocytes are part of the major components of the adaptive immune response against pathogens (Murphy, 2012).

Therefore, toxicity from some chemicals used in the paint production could possibly cause the slight alterations in the haematological indices of the wistar rats due to prolonged exposure to paints fumes.

CONCLUSION

Going by the findings obtained in the present study, prolonged exposure of the wistar rats to the paints fumes can cause some alterations in the full blood found count of the experimental animals. This suggest that occupational toxicity as a result to exposure to hazardous chemicals is common in industries including paint industries and paints exposed to organic and inorganic materials.

RECOMMENDATIONS

1. Further research should be carried out to discover suitable additives that could enhance the resistance of the extracted plant pigments to ultra Violet rays after incorporation in paint samples.
2. More research findings are needed on the body weight, skin and lungs histopathology to ascertain the effect of organic and inorganic colourants in paints on these organs due to prolonged inhalation of the paint fumes.

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