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# Investigation on the Compatibility of Extracted Plant Pigments in Emulsion Paints and the Effects of Inhalation on the Liver Enzymes of Wistar Rats

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**Annotation:** The compatibility of Curcuma longa rhizome and Hibuscus sabdariffa flowers pigment extracts in white emulsion paint and the effect of inhalation on the liver enzymes of Wistar rats were investigated. 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

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 Wistar rats in each group were exposed to the different paint sample through inhalation. The result revealed that there was a significant increase ($p<0.05$) in the serum ALT activity of the Wistar rats in Group E exposed to 2mls C. longa and inorganic yellow oxide paste in white emulsion paint from 1st to 6th month of exposure. However, Group G also showed increase serum ALT at 4th and 6th month of exposure compared to the normal control group H. Significant increase was also recorded in the serum AST activity of Group B, C, D, E, F and G from 2nd to the 6th month of exposure compared to the normal control group H. Also, the serum ALP activity of Group A, B, C, D, E, F and G recorded significant increase from the 1st to the 6th month of exposure compared to the normal control group H. Therefore, inhalation of the paint fume by the Wistar rats elicited significant alterations in the concentration of ALT, AST and ALP. This indicated that paint chemicals can adversely affect the liver enzyme due to prolonged inhalation of the paint fume by the Wistar rats.

**Keywords:** Curcuma longa, rhizome, Hibiscus sabdariffa, haematological parameters, toxicity.

## INTRODUCTION

Paints are homogeneous liquids or mixtures that, when applied to a substrate, solidify and dry to create a continuous, smooth hard layer. It can stick to the substrate, or intended surface, whether applied with a hand brush, paint roller, or spray gun. It might be a physical process (loss of solvent, for example) or a chemical process (low or high temperature polymerization, also known as drying, curing, or cross linking). The three main ingredients of paints are binders, pigments, and solvents. Additives are a category for other specialized materials. (Karakas *et al.*, 2015).

Water-based and oil-based coating systems are the two broad categories into which paints fall. The primary solvent or lubricant in water-based paints is water, but the solvent in oil-based paints can

be hydrocarbon, aromatic, ester, etc. (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. Depending on the dispersion stage of the production process, colorants are typically added in the form of paste or pigment during the color matching, tinting, or pigment grinding processes. In the meantime, the pigments or colorants are the only factors that determine the paint's final color. Additionally, they are entirely in charge of the paint's opacity and light fastness after application (Fernandez and Depablo, 2022).

Various grades of hazardous chemicals found in paint materials, such as solvents (aromatic hydrocarbons including benzene, toluene, and xylene), are exposed to paint workers (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 pigments (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).

In general, inorganic pigments in paints are more stable against sunlight than their organic counterparts; however, the former's colors and hues are often duller than the latter's sharper and brighter tones. As a result, a combination of both organic and inorganic pigments is required to get certain desirable colors for decorative purposes. Pigments are found naturally in many fruits and vegetables.

Chlorophylls (green), carotenoids (yellow, orange, red), betalains (orange), and anthocyanins (red-blue, purple) are the four primary chemicals that have distinct properties (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, anthocyanins, betalains and carotenoids have extensive use for red, orange and yellow shades respectively (Da-costa *et al.*, 2014).

Nowadays, it is normal practice to employ plants like *Hibiscus sabdariffa* and *Curcuma longa* rhizome to improve the qualities of paint products and lessen the harmful effects of the raw materials used to make indoor and outdoor paints. Because of the country's hyperinflation, almost all raw ingredients, including pigments and colorants, which are essentially imported, are extremely expensive. As a result, the finished paints are out of the price range of even the low income end consumers.

Thus, the purpose of this study was to examine the coloring potential of the plant extracts, Hibiscus sabdariffa and Curcuma longa rhizome, as well as their compatibility and resistance to UV rays in water-based paints. Additionally, the study sought to determine the effects of prolonged inhalation (due to exposure) on the red and white blood cells 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, where 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 form using corona manual grinding machine. Exactly one (1kg) each of the dried pulverized *Curcuma longa* and *Hibiscus sabdariffa* were separately soaked in 5mls 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 Whatman 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 of 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 at 1:1 ratio. The twenty milliliters of each paint sample 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 to ensure continuous exuding of fumes and prevent it from drying.. All animals were allowed free access to commercial rat mash and water throughout the six months of experiment. Good ventilation and hygiene were strictly maintained by constant cleaning, removal of faeces and spilt feeds from the cages daily.

## COLLECTION OF BLOOD SAMPLE AND PRESERVATION OF SERUM

After every one month interval, blood samples were collected from the eyes of the Wistar rats ocularly, the blood samples were centrifuged to separate the serum used for determination of the concentration of liver enzymes. After 6 months of exposure to paint fumes and feeding, the animals were subjected to overnight fast, then they were anaesthetized with chloroform vapour and were sacrificed by dissecting medioventrically and the blood sample collected through cardiac puncture by means of syringe and needle into well labeled anticoagulant (EDTA) bottles and gently shaken and allowed to stand for 1 hour after which they were centrifuged at 4,000RPM for 10minutes to separate serum from the blood cells.

The serum obtained was used for the determination of liver enzymes (ALT, AST and ALP) concentration.

## DETERMINATION OF SERUM ALANINE AMINO TRANSFERASE (ALT) LEVEL (ALANIN TRANSAMINASE)

This test was determined using the method of Reitman and Frankel (1957). ALT is measured by monitoring the concentration of pyruvate hydrazine formed with 2, 4-dinitrophenylhydrazine.

a-oxoglutarate + L-alanine GPT → L-glutamate + Pyruvate.

To two test tubes labeled blank and sample, 0.1ml of distilled water, 0.1ml of serum samples was measured into each tube respectively and 0.5ml of the substrate reagent (R1) was added to each of the test tubes which was mixed and incubated for 30 minutes at 37°C. Then 0.5ml of 2, 4-dinitrophenylhydrazine solution (R2) was mixed and allowed to stand for 20 minutes at 20-25°C and finally 5ml of sodium hydroxide (0.4ml) was added to each tube, mixed and the absorbance of the sample (A sample) was read against the reagent blank after 5 minutes using spectrophotometer at 546nm, and the concentration was obtained.

## DETERMINATION OF SERUM ASPARTATE AMINO TRANSFERASE (AST) LEVEL (ASPARTATE TRANSAMINASE)

This test was done using the method of Reitman and Frankel (1957). This principle is based on transamination reaction between L-aspartate and L-glutamate catalyzed by AST. It is measured by monitoring the concentration of oxaloacetate hydrazine formed with 2, 4-dinitrophenylhydrazine.

a-oxoglutarate + L-aspartate GoT → L-glutamate + oxaloacetate.

To two test tubes labeled blank and sample, 0.1ml of distilled water and 0.1ml of Serum samples was measured into the test tubes respectively, and 0.5ml of the substrate reagent (R1) was added to each of the test tubes which was mixed and incubated for exactly 30 minutes at 37°C. Then 0.5ml of 2, 4 dinitrophenylhydrazine solution (R2) was mixed and allowed to stand for 20 minutes at 20-25°C and finally 5 ml sodium hydroxide (0.4ml) was added to each tube, mixed and the concentration was obtained by reading the chart provided by the manufacturer.

## DETERMINATION OF SERUM ALKALINE PHOSPHATASE (ALP) LEVEL

Alkaline Phosphatase in serum was determined using the method described by Englhant (1970), using Randox laboratory kit which employs the fixed procedure for the determination of enzymes.

p-nitrophenylphosphate + $H_2O$ ALP → phosphate + p-nitrophenol.

To three test tubes labeled blank, sample and control, 0.05ml of distilled water, 0.05ml of standard and 0.05ml of the control sera was measured into each test tube respectively. Then, 0.05ml of n-nitrophenylphosphate substance was mixed and incubated for 10 minutes at 37°C. Thereafter, 2.5ml of Alkaline phosphatase colour developer was added to each test tube and mixed.

Absorbance was read using spectrophotometer at wavelength of 590nm. Concentration of samples were calculated using:

Absorbance of sampleAbsorbance of standarad x concentration of standard1

## 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

The liver function parameters analyzed to check the effect of long-term exposure to different sample of both organic and inorganic colourants in white emulsion paint include alanine transaminase (Figure 1), aspartate transaminase (Figure 2), alkaline phosphatase (Figure 3). The

bar-charts indicated in each figure represents the ALT, AST and ALP parameters for a period of six months analysed at monthly interval.

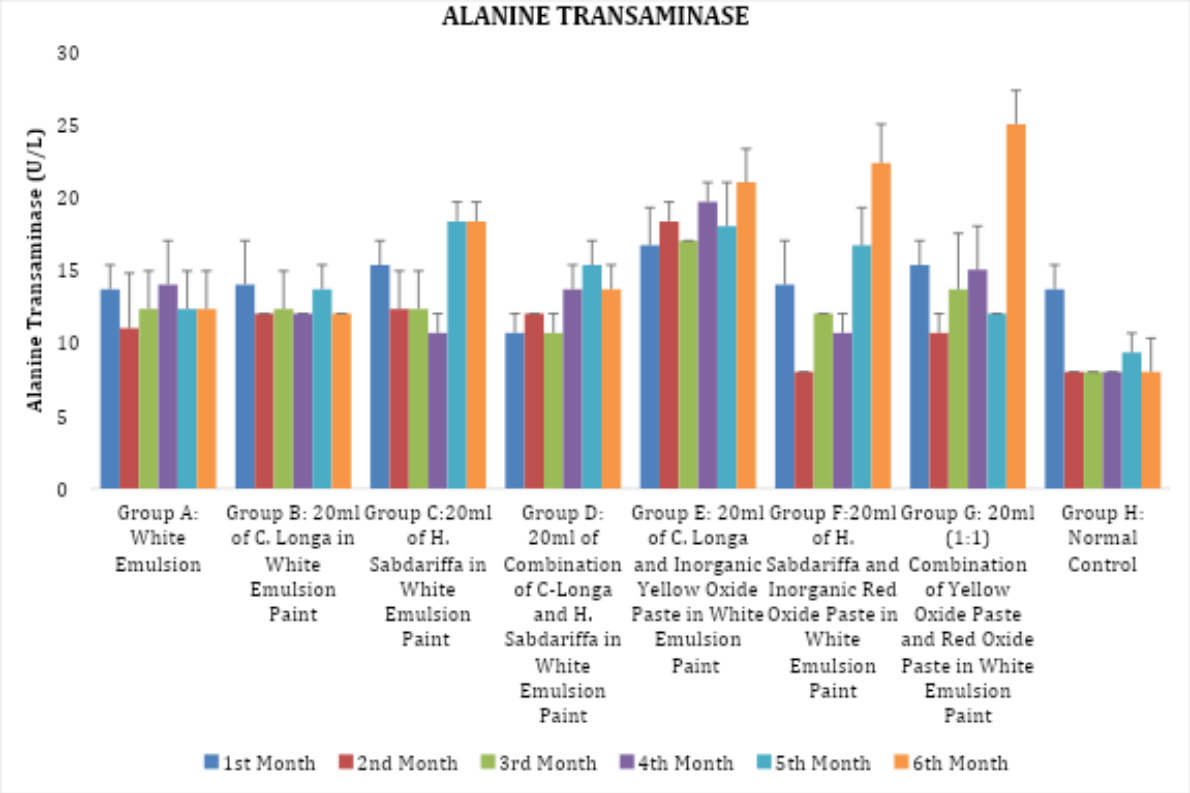


**Figure 1:** Results of the effect of *C. longa* and *H. sabdariffa* mixed with paint on alanine transaminase of Wistar rats.

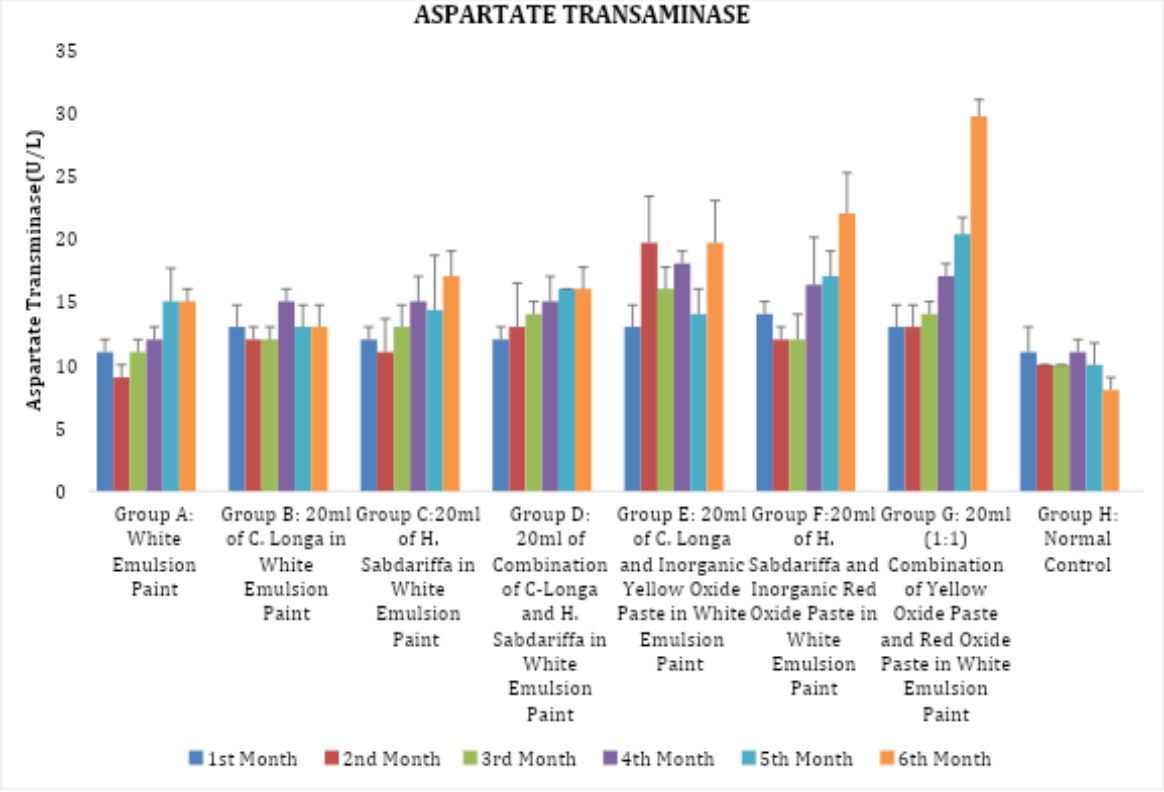


**Figure 2:** Results of the effect of *C. longa* and *H. sabdariffa* mixed with paint on aspartate transaminase of Wistar rats.

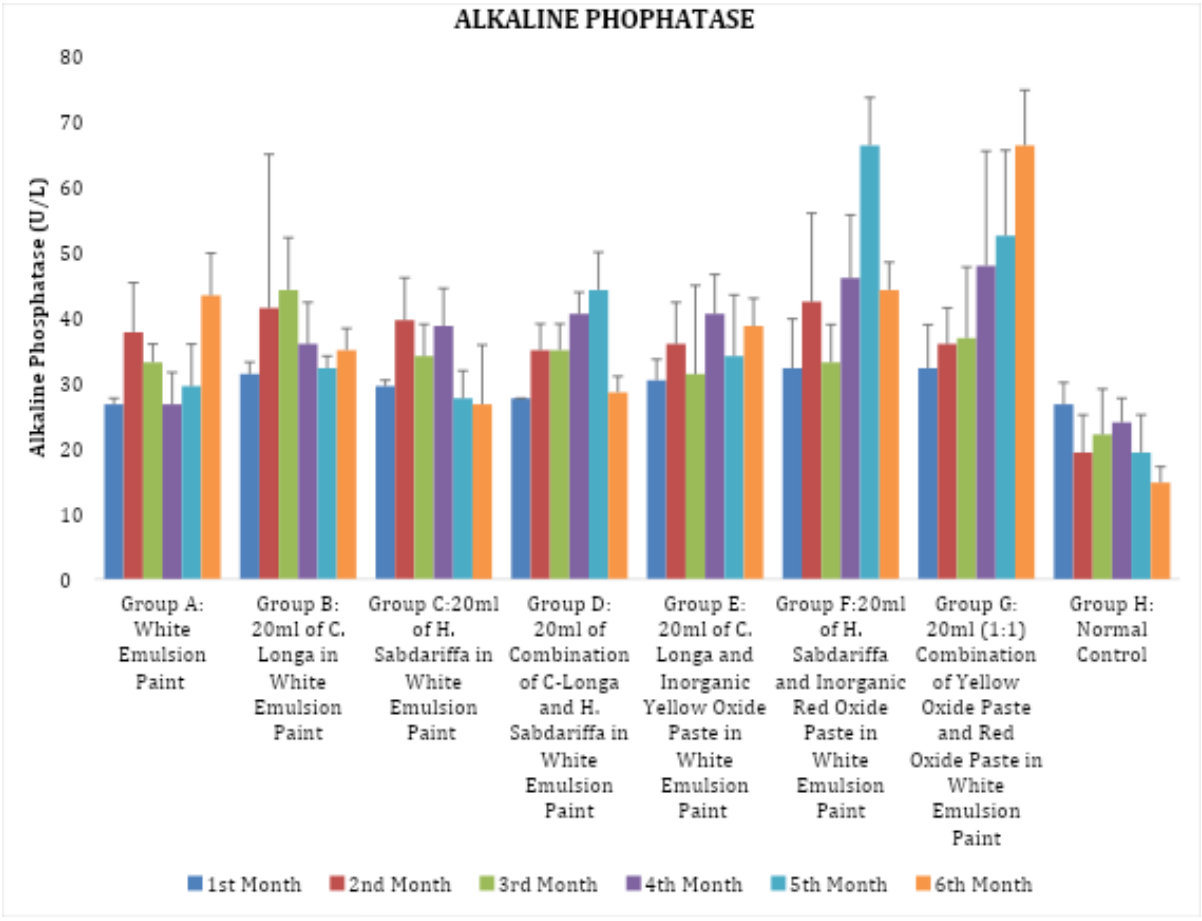


**Figure 3:** Effect of *C. longa* and *H. sabdariffa* mixed with paint on alkaline phosphatase of Wistar rats.

## DISCUSSION

The result obtained from the analysis revealed that the alanine transaminase (ALT) activity of the different groups did not increase significantly within the first two months of the experiment. Meanwhile, there was a significant increase ($p<0.05$) in the serum ALT activity of group A, E and G from second month of the experiment compared to normal control group H. A significant increase ($p<0.05$) in serum ALT was also observed in group B, C, D and F on the fifth month of the experiment compared to the normal control group H. The observed significant elevated serum ALT liver enzyme serves as a biomarker for the prediction of liver toxicity and as such has been used in scientific report. This result was in conformity with the work of Gupta, (2005) who reported that dichlorvos caused hepatoxicity in rats leading to oxidative stress. Also, Helal, (2011) opined that long period dichlorvos inhalation can change plasma prooxidant-antioxidant balance, and thereby advised the need to be cautious of long term exposures.

Meanwhile, the normal range for ALT in adults is 5-40 IU/L. Therefore, increased concentration of ALT in the blood is indicative of liver disease or damage because it occurs exclusively in the liver and it is markedly increased in liver cirrhosis and liver cell necrosis (Uboh *et al.*, 2010).

However, elevated serum ALT in the different groups as the months progressed from second to sixth month showed toxic potential of the paint fumes due to prolonged inhalation.

The aspartate transaminase (AST) activity of the different groups did not increase significantly ($p>0.05$) within the first three months of the experiment. However, a significant increase ($p<0.05$) in the AST activity of the organic and inorganic colourants in white emulsion paint became well noticeable from the fourth to the sixth month of exposure. Meanwhile, there was a significant increase ($p<0.05$) in the AST activity of groups C, D, E, F and G on the 4th, 5th, and 6th months of the experiment compared to the normal control group H. The increase in the concentration of

AST in the different groups could not be attributed to liver damage because AST and ALP enzymes originate from different tissues such as liver, bone, intestine and pancrease. Therefore, the increase might emanate from other sources than the liver cell (Odutola, 2014). However, the normal range for serum AST in adults is 5 – 45 IU/L (Bedi *et al.,* 2016)

The result of ALP revealed significant increase in Group B, D and F in the second month compared to normal control. A significant increase in the ALP concentration in group B was noticed in the third month compared to the normal control. Also, group D, E, F and G showed a significant increase in the level of ALP in the fourth, fifth and sixth months compared to the normal control group H and the normal range (30 – 130 IU/L).

The result of this study conformed with the argument of Sharma and Singh (2012), who reported that a volatile chemical such as dichlorvos caused oxidative stress in rats through abnormal production of reactive oxygen species. Belquet (2010) also reported that the main mechanism of liver damage is by bioactivation of reactive oxygen species and free radicals that elicit oxidative stress. However, it is possible that the elevated ALP could have originated from other different tissue aside from the liver.

## CONCLUSION

Going by the results obtained in this study, it may be concluded that the elevated concentrations of ALT, AST and ALP above the homeostatic limits in various groups as the months of exposure of the Wistar rats progressed towards the sixth month, could be attributed to some forms of disorders which may affect the functional integrity of the liver cells and tissues.

### Recommendations

1. Workers should be educated on work related hygiene and hazards involved in the use and direct inhalation of fumes from organic and inorganic colourants used in the manufacturing of emulsion paints.
2. Further study can be carried out on the effects of prolonged inhalation of the extracted plant pigments in emulsion paints, on other Biochemical parameters and organ- system function of Wistar rats.

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