Hepatic Effects of the Dermal Absorption of Emulsion Paint Solution by Albino Rats

*Igwe, F.U., *Wosu, H.W., **Ogunka-Nnoka, C.U.

*Department of Biochemistry, Faculty of Science, Rivers State University of Science and Technology, Port Harcourt, Nigeria.

**Department of Biochemistry, Faculty of Science, University of Port Harcourt, Choba, Port Harcourt, Nigeria. Corresponding Author: igwe.felix@ust.edu.ng Mobile +234 803 310 7945

Abstract: Paint solutions are admixture of different chemical compounds commonly used for colouring and protecting many surfaces in houses, cars, factories, equipment, vessels and so on. Painters and paint factory workers are constantly exposed to components of paint solutions through inhalation and dermal contact. The hepatic effects of dermal absorption of an emulsion paint solution by male albino rats were evaluated. The albino rats were exposed to sub lethal dose of the paint solution by intraperitoneal method every 48 hours for 14 days. Sets of the rats were weighed and sacrificed after 7 days and 14 days treatment respectively and their blood collected for serum alanine transaminase (ALT), aspartate amino transaminase (AST) and alkaline phosphatase (ALP) enzyme assay by spectrophotometric method. The liver was also excised, weighed and prepared for Total protein, Albumin and Histopathology analyses using standard methods. Body weight and corresponding wet liver weight of the treated rats decreased significantly ($p<0.05$) during the treatment period when compared with controls. Levels of serum ALT, AST and ALP in the treated rats also decreased significantly ($p<0.05$) when compared with controls. Liver Total proteins and Albumin contents decreased significantly ($p<0.05$) after 7 days treatment and increased significantly ($p<0.05$) after 14 days treatment when compared to controls. Histological examination of the liver tissue of the control group showed normal architecture whereas hepatocytes of the groups treated with the paint solution were congested with hepatocellular distortion and microvesicular steatosis. These results suggest that continuous dermal exposure to emulsion paint solution may be hepatotoxic and capable of eliciting systemic toxicity.

Key Words: Dermal exposure, Emulsion paint, Hepatic effects, Histological examinations, Liver enzymes.

INTRODUCTION

Toxicity studies in animals are commonly used to access potential health risk in humans caused by intrinsic adverse effects of chemical compounds [1].

Paints are any liquid or mastic composition that converts to a solid film after application to a substrate in a thin layer. They are one of the oldest synthetic substances known in the history of mankind stretching back into prehistoric times. A typical paint is a complex mixture composed of pigments, extenders, binders, solvents and other additives [2]. The binder, a polymeric substance that is either dissolved in the continuous phase of the paint or suspended in it by emulsifiers, is the film-forming component of paints that imparts properties like gloss, durability, flexibility and toughness to the paint [2]. Binders include synthetic or natural resins such as alkyd resin, acrylics, vinyl-acrylics, polyurethanes and epoxy resin. Whereas alkyd resins like polyesters are the most common resins used in solvent based paints, vinyl-acrylics and acrylic emulsions are the emulsions-in-water most commonly used as binders in water based household paints [2]. Paint solutions are also reported to contain some toxic heavy metals [3].

The liver, located behind the ribs in the upper right-hand portion of the abdomen, is one of the largest organs in mammals. It is a remarkable organ able to regenerate, repair or replace injured tissues effectively. As part of the biliary system, it is exposed to many potentially harmful substances [4]. Its structure is such that many lobules perform the same task, therefore, if one section of the liver is damaged, another section will perform the functions of the injured area indefinitely or until the damaged section is repaired. However, the liver is subject to many conditions that can overwhelm its regeneration ability, thus threatening overall health. Drug use, including long term use of some prescription medications as well as illegal drugs, addiction usage, prolonged consumption of concoctions and smoking, may lead to liver damage [5]. As the central processing unit for metabolism of exogenous substances, the liver functions to produce immune factors that removes toxicants and other xenobiotics from the blood stream to combat infection. The liver also controls the production and removal of cholesterol, makes clotting factors to stop excessive bleeding, clears the blood of waste products, drugs, and other poisonous substances as well as stores vitamins, sugar and iron to help in metabolism [6]. Hepatocytes, which make up 70 – 80% of the cytoplasmic mass of the liver, are involved in the protein, carbohydrates and lipids metabolism, bile formation and secretions, detoxification, modifications and transformation of exogenous and endogenous substances [6]. The liver plays a central role in transforming and clearing chemicals and is therefore susceptible to the toxicity from these agents. Exposed chemical substances may experience detoxification and inactivation in the liver and become less harmful to the system [7] or hepatic cell damage can occur as a result of metabolism through the process of reduction, oxidation, hydroxylation and conjugation [7]. Abnormalities associated with liver function can be seen from increased serum activities of the enzymes alanine transaminase (ALT), aspartate amino transaminase (AST) and alkaline phosphatase (ALP) which may indicate liver cell damage or hepatocellular necrosis [3].

Most painters and paint factory workers are in constant contact with paint solutions and the chemicals contained therein although the patterns and levels of exposure to individual agents may differ [8]. Toxicity can occur from acute unintentional or deliberate exposure to excessive amount of paint fumes, ingestion or trans dermal absorption which may results in serious multi-organ toxicity and death [9]. Occupational exposure to paint solutions by way of inhalation, ingestion and skin contact have produced undesirable effect such as sneezing, increase in salivation, skin erythema, nausea, vomiting, photophobia, disorientation, diplopia, ataxia, speech disorders and decrease in reflexes [10]. Chronic use of paint solutions have been reported to be capable of causing permanent damage to the central nervous systems, liver, kidney, heart and lungs [10]. Lead, cadmium, nickel and other heavy metals used as part of paint solutions have also been reported to have adverse health implications [3]. According to Afolayan *et al* [1], adverse effects may manifest as significant alteration in the levels of biomolecules such as enzymes, metabolic products, normal functioning and histomorphology of the organs. This work investigated the effects of dermal exposure of an emulsion paint solution on the hepatosomatic index, serum liver function enzymes activity as well as liver protein levels and histology of adult male albino rats.

MATERIALS AND METHODS

Sample Source

The emulsion paint solution was obtained from the factory of a paint manufacturing company located in Port Harcourt, Rivers State, Nigeria and brought to the laboratory. The paint solution was stored at room temperature in its original plastic container before use.

Animal Treatment Protocol

A total of 20 male albino rats weighing 120 -150g were obtained from the animal house of the Department of Biochemistry, University of Port Harcourt and transferred to the Biochemistry unit of the Department of Chemistry, Rivers State University of Science and Technology, Port Harcourt, Nigeria. All animals were housed in separate metal cages and maintained at room temperature and an alternating 12-hour light-dark cycle. They were given access to standard rodent feed and water *ad libitum* and acclimatized to the laboratory environment for 7 days before being randomly assigned to four groups of five rats in each group. Two separate groups were designated as "Control" (C_7 and C_{14}) while the other two groups were designated as "Treated" (T_7 and T_{14}). The Treated groups were administered with 2.5mg/kg body weight of the paint solution at the dorsal skin area by intraperitoneal injection every 48 hours and the Control groups were left untreated. Both groups (Treated and Control) were fed *ad libitum* with the standard rodent feed and water. The investigation was carried out based on the approved institutional guideline for the care and handling of laboratory animals. The animals were treated in accordance with National and institutional guidelines for the protection of animal welfare during experiments [11].

Sample Collection

At the end of the first 7 days of treatment two groups (C_7 and T_7) were weighed and sacrificed by cervical dislocation. Their blood samples were collected from the portal vein into dry clean heparin bottles and left at room temperature to clot. Afterwards they were centrifuged at 300 rpm for 15 minutes. Separated serum was carefully aspired and transferred into dry clean heparin bottles stored at 5^0C for enzyme assay. The rats were further dissected and their liver carefully excised, cleaned of any attached fatty strands and weighed. Part of the weighed liver was processed for histopathology while the other part was homogenized in ice-cold phosphate buffer for Total protein and Albumin analyses. At the end of 14 days of treatment the remaining groups (C_{14} and T_{14}) were weighed, sacrificed and processed for analyses as applicable to C_7 and T_7.

Biochemical Assay

Serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) as well as liver Total protein and Albumin content were determined by spectrophotometric method using the respective Randox test kits [12] and following the manufacturer's instructions. Liver Total protein and Albumin content were determined from the liver homogenate. To prepare the homogenate, the excised liver was quickly transferred into ice cold phosphate buffer of pH 7.2 and homogenized. A 1:10 liver: phosphate buffer solution ratio was utilized to homogenize the liver using a homogenizer immersed in an ice bucket. The homogenate was filtered with Whatman No. 1 filter paper and the clear filtrate collected for the Total protein and Albumin analysis.

Histopathology Investigation

A portion of the excised rat liver was fixed in 10% formalin, washed, dehydrated in isopropanol, rinsed with xylene and embedded in paraffin wax. The paraffin sections were prepared and stained with haematoxylin and eosin. Thin sections of the liver was made into permanent slides and examined under microscope with photographic facility and photomicrographs were taken. The micrographs were analyzed and interpreted by the pathologist. The processing, sectioning, staining, microscopic examination and interpretation were carried out as described by [13]

Statistical Analysis

Results from the investigation were expressed as mean of at least triplicate determinations ± standard deviation. Comparison between treated and corresponding control were made using student's t- test. A value of $p<0.05$ was considered as statistically significant [14]

RESULTS

Relative Liver Weight

Results of the body weight, liver weight and liver to body weight ratio (hepatosomatic index or relative liver weight) are shown in Table 1. Body weight decreased by 49.20%, liver weight decreased by 41.25% and liver-to-body weight ratio increased by 13.56% among treated rats after 7 days exposure when compared to controls. 65.70%, 69.86% and 12.31% decrease in body weight, liver weight and liver-to-body weight ratio respectively were observed among the treated rats after 14 days exposure compared to controls

Table 1: Body and liver weights of albino rats exposed to paint solution

Parameters	7-days Control	7-days Treated	14-days Control	14-days Treated
Body Weight (g)	150.0 ± 7.07	76.2 ± 7.12[a] (-49.20)	193.0 ± 0.37	66.20 ± 2.67[a] (-65.70)
Liver Weight (g)	7.66 ± 0.14	4.50 ± 0.17[a] (-41.25)	12.54 ± 0.80	3.78 ± 0.58[a] (-69.86)
Relative Liver Weight	5.10 ± 0.34	5.90 ± 0.40[a] (13.56)	6.50 ± 0.37	5.70 ± 0.20[a] (-12.31)

Values are mean of five different rats in each group ± standard deviation. Values in parenthesis are % change between control and treated groups. [a] represent values that are statistically significant ($p<0.05$)

Serum Liver Function Enzymes

The results of serum liver function enzymes activity are shown in Table 2. There was significant ($p<0.05$) decrease in the activity of serum AST, ALT and ALP in the treated rats compared to controls. AST, ALT and ALP activity decreased by 15.97%, 37.85% and 33.97% respectively after 7 days exposure and 1.53%, 44.48% and 39.19% respectively after 14 days exposure.

Table 2: Serum liver function enzyme activity of albino rats exposed to paint solution

Enzymes Activity (IU/L)	7-days Control	7-days Treated	14-days Control	14-days Treated
AST	118.17 ± 1.82	99.30 ± 1.23[a] (-15.97)	109.17 ± 10.65	107.50 ± 21.56 (-1.53)
ALT	34.00 ± 0.17	21.13 ± 0.67[a] (-37.85)	42.20 ± 2.73	23.43 ± 1.21[a] (-44.48)
ALP	196.33 ± 4.91	129.63 ± 4.62[a] (-33.97)	205.00 ± 23.18	124.67 ± 14.90 [a] (-39.19)

Values are mean of five different rats in each group ± standard deviation. Values in parenthesis are % change between control and treated groups. [a] represent values that are statistically significant ($p<0.05$)

Liver Total Protein and Albumin Content

The Total protein and Albumin content of the rat liver homogenate are shown in Table 3. After 7 days exposure, Total protein content of the treated rats decreased significantly ($p<0.05$) by 14.06% and Albumin content decreased significantly ($p<0.05$) by 24.30% compared to controls. However, after 14 days exposure Total protein content increased significantly ($p<0.05$) by 29.18% and Albumin content increased significantly ($p<0.05$) by 27.59% amongst the treated rats compared to controls

Table 3: Liver Total protein and Albumin content of albino rats exposed to paint solution

Parameters	7-days Control	7-days Treated	14-days Control	14-days Treated
Total Protein (g/dL)	42.67 ± 1.86	36.67 ± 0.33 [a] (-14.06)	31.16 ± 1.61	44.00± 2.51 [a] (29.18)
Albumin (g/dL)	35.67 ± 0.67	27.00± 1.15 [a] (-24.30)	21.00 ± 0.58	29.00± 0.48 [a] (27.59)

Values are mean of five different rats in each group ± standard deviation. Values in parenthesis are % change between control and treated groups. [a] represent values that are statistically significant ($p<0.05$)

Histopathology Investigation

Histomicrographs of the liver of albino rats used in the study are contained in Figures 1, 2, 3 and 4. Whereas the rats treated with paint solution for 7 days showed congested hepatocytes with hepatocellular distortion (Figure 1) and the rats treated with paint solution for 14 days showed congested hepatocytes with microvesicular steatosis (Figure 3), the 7 days and 14 days controls showed normal cellular architecture (Figures 2 and 4 respectively)

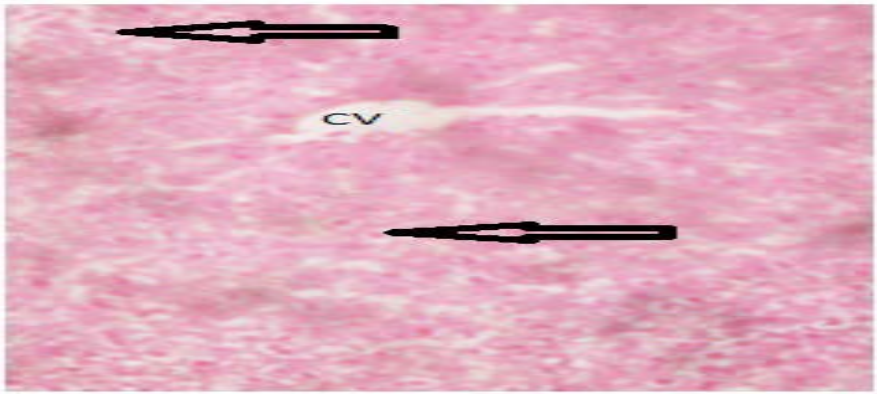

Figure 1: Histomicrograph of rat liver treated with paint solution for 7days showing congested hepatocytes (arrow-marked) with hepatocellular distortion. The part marked CV is the central vein

Figure 2: Histomicrograph of 7days control rat liver showing normal cellular architecture. The part marked CV is the central vein.

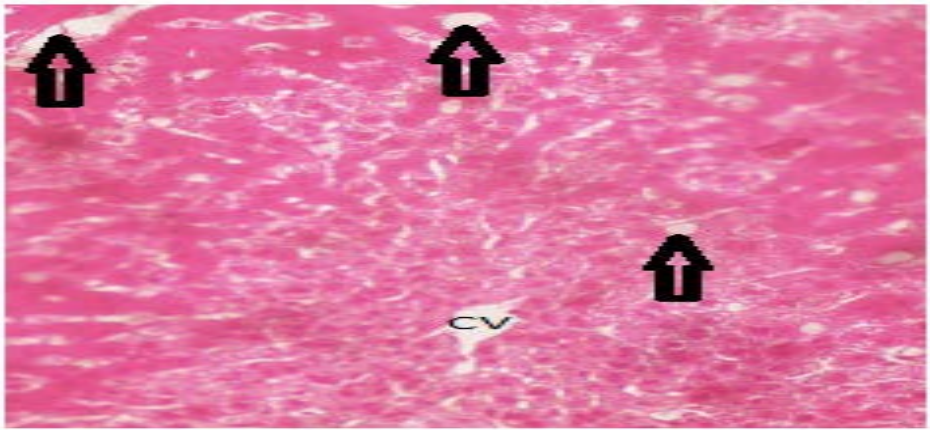

Figure 3: Histomicrograph of rat liver treated with paint solution for 14days showing congested hepatocytes with microvesicular steatosis (arrow-marked). The part marked CV is the central vein

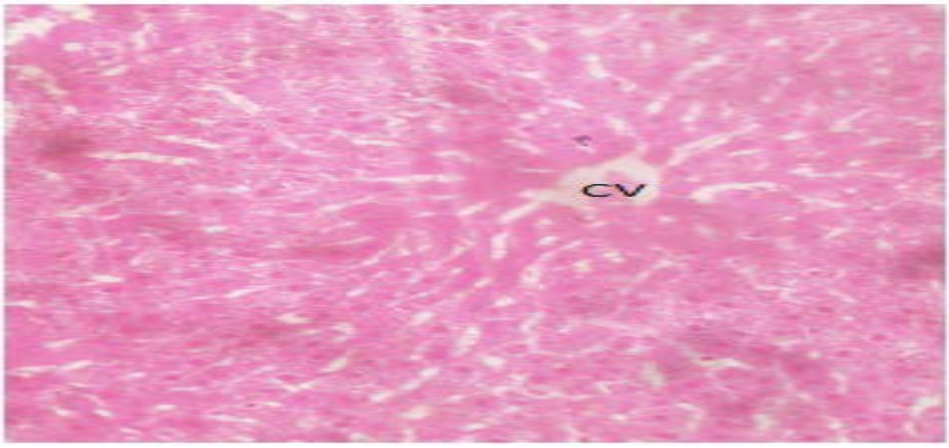

Figure 4: Histomicrograph of 14 days control rat liver showing normal cellular architecture. The part marked CV is the central vein.

Discussion

Organ weight changes are often associated with treatment-related effects and are widely accepted in the evaluation of test article-associated toxicities. The practice of weighing organs during toxicology studies are performed in pharmaceutical, animal health, chemical, food/nutritional and consumer product industries worldwide. Therefore the Society of Toxicologic Pathology (STP) recommends that organ weights be included routinely in multi dose general toxicity studies with durations from 7 days to 1 year [15].

In this study the significant reduction ($p<0.05$) in both liver weight and body weight of the rats exposed to paint solution implies an obvious toxic effect which interfered with essential metabolism associated with the liver. The significant ($p<0.05$) changes in hepatosomatic index (Table 1) further shows that cellular activities in the liver associated with the absorption of the paint solution had affected tissue growth and development.

Measurement of the activities of marker enzymes in tissues and body fluids can be used in assessing the degree of assault and the toxicity of a chemical compound on tissues and organs [16, 17]. Measurement of enzyme activities can also be used to indicate tissue cellular damage caused by a chemical compound long before histological changes become evident [18]. The results in this study showed that there was significant ($p<0.05$) reduction in AST, ALT and ALP in the 7 and 14 days treatment period compared to the requisite controls. Alkaline phosphatase (ALP) a marker enzyme for the plasma membrane and endoplasmic reticulum is frequently used to assess the integrity of the plasma membrane such that any alteration is evident in the activity of the enzyme in the tissue and serum [19, 18, 20]. The reduction in ALP activities following exposure to the paint solution may be adduced to alteration of membrane components integrity attributed to the toxicity induced by the paint solution. This reduction may be as a result of the loss of some membrane components (into extracellular fluid), inactivation of the enzyme molecule in such situation [21] and/or inhibition of the enzyme activity at the cellular/molecular level by the toxin. It may also be due to reduction in concentration or total absence of specific phospholipids required by this membrane bound enzyme to express its full activity [22]. This reduction in ALP activity is in line with the report of

Malbica and Hart [23] in which there was reduction in ALP activities following the administration of $KBrO_3$.

ALT is an enzyme that helps to metabolize the amino acid, alanine and it is normally present in blood at low levels. An increase in ALT levels may indicate liver damage, while decrease could be a result of inhibition of certain part of the transamination process in which the enzyme is involved. The decrease in serum ALT activity in this study could indicate that there is decrease in alanine metabolism in the liver which implies a hepatotoxic effect.

AST is an enzyme found in the liver that helps to metabolize proteins. When the liver is damaged, AST is released into the blood stream and its activity increases. The observed reduction in serum AST levels could be an indication that the enzyme is being inactivated or hepatic protein metabolism is reduced or inhibited as a result of the treatment. This reduction in serum AST and ALT activity is similar to the report in another study by Anofi *et al* [24].

Albumin is one of several proteins made in the liver. The system needs this protein to fight infection and to perform other functions such as transportation of materials in blood. Lower than normal levels of albumin may indicate liver damage or disease. The reduction in total protein and albumin level in the liver after 7 days treatment in this study could be an indication that the paint solution inhibited hepatic albumin and protein syntheses which could lower the immune response of the animals. The reduction in liver weight, body weight and AST levels corroborates this assertion. Anofi *et al* [24] had reported similar toxicity findings as well. The increased protein and albumin levels associated with the treated rats after 14 days exposure, when compared to controls, could be attributed to increased synthetic activities as the tissues regenerates to recover from the initial damage. This further indicates possible impairment in the normal function of the liver.

Histological examination of tissues could serve as complementary evidence to enzyme studies toward revealing any distortion or damage to the normal structure of the tissue cells. The congested hepatocytes observed following exposure to the paint solution may be due to damage induced by toxic chemical compounds in the paint. This is an indication of cirrhosis which usually disrupts the normal flow of blood through the liver [25]. The hepatocellular distortion and microvesicular steatosis observed after 14 days treatment shows how this possible toxic effect can be progressive as the duration of exposure increases. Liver injury due to drugs and chemicals may occur if the durability and liver regeneration ability is reduced. This can cause permanent liver damage that may have dangerous impact on the entire system.

CONCLUSION

The results of this study indicates that intraperitoneal administration of sub lethal dose of emulsion paint solution on adult male albino rats for 14 days period led to significant alterations in body weight, liver weight, hepatosomatic index, liver total protein and albumin content as well as serum liver function marker-enzymes. These effects may be attributed to possible disruption of liver cell membrane architecture and other inhibitory metabolic activities induced by the paint solution. Results of histological examination of the liver also showed that treated rats had congested hepatocytes with hepatocellular distortion and microvesicular steatosis. These findings suggest that continuous dermal

exposure of mammals to paint solution may be hepatotoxic with possible adverse systemic effects.

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