Hepatotoxicity effect of oral exposure to Nitrocellulose thinner in albino Wistar rats

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Abbreviations:
NCT: Nitrocellulose thinner, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma glutamyl transferase, ALP: Alkaline phosphatase

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Abstract
Nitrocellulose thinner (NCT) is one of the industrial solvents commonly used in furniture, painting and automobile spray painting industries. It has been reported to induce haematotoxicity and nephrotoxicity in experimental animals. This study assessed the effect of NCT on serum liver enzymes (including ALT, AST, GGT and ALP) activities, conjugated and total bilirubin, albumin, total proteins, and histopathology of the liver tissue in albino Wistar rats (140.0 ± 20.0 g). Varying concentrations (10, 15 and 20 mg/kg body) of NCT were respectively administered orally, once daily, to experimental rats for 30 days. The results showed a concentration-dependent significant increase (p ≤ 0.05) in serum liver enzymes activities, conjugated and total bilirubin; and decrease in serum albumin and total proteins concentrations, compared to control. Also, negative histopathological alterations were observed in the liver tissues of rats exposed to NCT. These biochemical and histopathological assays’ results gave a strong indication that exposure to 15 and 20 mg/kg body of NCT caused a significant adverse effect on the liver functions in rats. From this study, it may be concluded that oral exposure to NCT is a risk factor for hepatotoxicity, hence the need to use it under regulatory measures.

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1. Introduction
Nitrocellulose thinner is one of the industrial solvents commonly used in furniture, paints and automobile spray painting industries. Hence, furniture, paints and automobile spray painting workers may be considered to be among those that are frequently exposed to this solvent, occupationally. Nitrocellulose thinner solvent is known to contain different organic chemical agents, including ethylbenzene or toluene and butyl acetate. These chemical agents are known to constitute chemical pollutants in the environments where they are used. Particularly, they have been detected in household and workplace air (WHO, 1996; 2005). They are generally used in mixtures with other solvents in domestic or industrial products (e.g. varnishes). Thus, occupational exposure to mixtures of toluene, ethylbenzene and butyl acetate have been reported in workplaces involving painting or lacquering (Vincent et al., 1994; Muttray et al., 1995; Seeber et al., 1996; Jovanovic et al., 2004).
Occupational exposure to organic solvents may occur through different routes, including inhalation, oral ingestion, or topically. Particularly, inhalation is generally regarded as the most likely route of exposure for workers and the general population, due to the volatility of these chemicals. However, oral ingestion of the chemicals, accruing from their bioaccumulations in foods and drinks are also commonly possible. According to Khan et al. (2010), automobile workers are at high risk for lung, urinary tract, brain and skin cancers due to their direct exposure to polycyclic aromatic hydrocarbons and lead toxicity. Exposure to different organic solvents has been reported to cause adverse effects on the functional integrity of different tissues in the biological systems (Uboh et al., 2010; 2009; 2008; 2007; Saillenfair et al., 2007; 2006; 2003; Faber et al., 2006; Robert-Gnansia and Saillenfair, 2002). However, the role of the liver tissues in the metabolism of foreign organic chemical substances is known to make the liver one of the primarily target tissues to be affected by either these chemical substances, or their metabolites. Liver is considered the key organ in the metabolism, detoxification and secretory functions in the body. It is known to regulate various important metabolic functions. Exposures to various chemical agents have been reported to alter the functional integrity of the liver tissues (Rezg et al., 2006; 2007). Among the chemical agents reported to be hepatotoxic include such industrial solvents as carbon tetra chloride, toluene, ethylbenzene, butyl acetate, organophosphates and organochlorides (Seeber et al., 1996; Jovanovic et al., 2004; Rezg et al., 2006; 2007; Prakash et al., 2008). Hepatic damage is associated with distortion of these metabolic functions. This present study therefore aimed at investigating the effect of nitrocellulose thinner, at varying concentrations, on the functional integrity of the liver tissues.

2. Objective of Research

Objective of this research is to assess the hepatotoxic effect of nitrocellulose thinner in rat model.

3. Materials and Methods

The widespread use of various industrial solvents, and the attendant health effects have been a concern to environmental and occupational health, as well as Toxicology researchers in recent times. Some of these solvents have been reported to induce tissue toxicities in both humans and experimental subjects. Nitrocellulose thinner is one of such industrial solvents that is commonly used in different societies today, and different individuals are frequently exposed this solvent either occupationally or in the course of domestic use. Its haematotoxic and nephrotoxic effects have been reported in experimental animals in our previous studies. This study was therefore designed to determine whether oral exposure to different concentrations, ranging from 10 mg/kg body weight, of nitrocellulose thinner may induce hepatotoxicity in experimental animals. It is hoped that the results of this study will contribute the basis for the regulation of the use of this solvent in our societies.

3.1 Chemicals

The laboratory reagent kits from Biosystems Laboratories (S. A. Costa Brava, Barcelona, Spain) were used to assess the activities of ALT, AST and ALP in the serum. While reagent kits from Randox Laboratories (United Kingdom)

3.2 Animals handling and treatment

Twenty four mature albino Wistar rats, weighing between 120 to 160 g were obtained from Biochemistry Department Experimental Research Animal House of the University of Calabar, Calabar, Nigeria. They were fed with a standard laboratory diet and tap water. Illumination was 12 hours light/dark cycle and room temperature was 25 ± 2°C. The animals were divided into four groups, [i.e., one control (A) and three experimental (B, C, D) groups], which consisted of six apparently normal albino Wistar rats per group. The experimental groups B, C and D were exposed daily to 10, 15 and 20 mg per kg body weight, respectively, of nitrocellulose thinner
(solubilized in cholesterol-free vegetable oil as a vehicle) by oral administration for 30 days, while the control group was given distilled water and equal volume of the vegetable oil used as vehicle for nitrocellulose thinner administration. The nitrocellulose thinner fraction administered was solubilized in Grand pure soya oil, obtained from Grand Cereals & Oil Mills Ltd, Jos, Nigeria. In this study, all animal experiment followed the Guidelines for the care and use of laboratory animals obtained from the Institutional Animal Ethics Committee.

3.3 Collection and preparation of blood specimen for analyses
Blood samples for bioassays were obtained from rats by cardiac puncture, under chloroform vapour anaesthesia, after 48 hours of termination of nitrocellulose thinner administration into sterile plain screw-cap sample bottles. The blood samples collected were allowed to clot, and the sera separated with Pasteur pipette, after spinning with MSE model (England) table-top centrifuge at 3000 rpm for 10 minutes. The separated serum samples were used for biochemical analyses. All biochemical analyses were carried out within 24 hours of serum separation.

3.4 Biochemical analyses
Biochemical analyses carried out included measurement of the activities of alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), as well as the levels of albumin, total protein, total and the conjugated bilirubin in the serum. Determination of the activities and concentrations of these biochemical parameters were done by spectrophotometric determination of their absorbances, using analytical grade laboratory reagent kits. The laboratory reagent kits from Biosystems Laboratories (S.A. Costa Brava, Barcelona, Spain) were used to assess the activities of ALT, AST and ALP in the serum. While reagent kits from Randox Laboratories (United Kingdom) were used to assess the activity of GGT, as well as the concentrations of albumin, total protein, total and the conjugated bilirubin in the serum. All absorbance readings were taken with DREL3000 HACH model spectrophotometer.

3.5 Histopathological studies of rat liver
After the animals were sacrificed, postmortem examination was performed and the rat livers were identified and carefully dissected out en bloc for histopathological examination. After rinsing the dissected liver in normal saline, tissue slice sections were taken from the liver organ. The tissue was fixed in 10% formo-saline, dehydrated with 100% ethanol solution and embedded in paraffin. It was then processed into 4-5 µm thick sections stained with haematoxylin-eosin and observed under a light microscope (Model N-400ME, CEL-TECH Diagnostics, Hamburg, Germany).

3.6 Statistical Analysis
Results were presented as mean ± S.E.M of six observations for each group. Statistical analysis was made using two-ways analysis of variance (ANOVA), using SPSS window statistical software programme. The values were considered significantly different when the P-value was less than 0.05.

4. Results
The results of this study are shown in Tables 1 and 2, as well as Figures 1, 2, 3, 4 and 5. These results showed that the serum activities of ALT, AST, GGT and ALP, as well as the levels of conjugated and total bilirubin obtained for the experimental test rats treated with 10, 15 and 20 mg/kg nitrocellulose thinner, in comparison with the control values, were significantly (p<0.05) higher, while the levels of albumin and total protein in the serum were statistically lower (Tables 1 and 2). However, while the percentage increase in the activities of serum ALT and GGT, at the different nitrocellulose thinner concentrations, were observed to be significantly higher than the increases in the activities of AST and ALP, the activity of serum ALP recorded to the lowest percentage increase (Figure 1a). The enormous increase in serum ALT and GGT activities than AST and ALP give a strong indication that liver tissue damage is associated with exposure to nitrocellulose thinner. Moreover, the percentage decrease in the serum albumin levels, at the different nitrocellulose thinner concentrations, were noted to be significantly higher than the respective percentage decreases obtained for total serum protein; suggesting inefficiency in the synthesis of albumin by the liver tissues. Similarly, the percentage increase in total bilirubin, at the respective nitrocellulose thinner concentrations, were significantly higher than the respective percentage increases obtained for the conjugated bilirubin (Figure 1b); this also suggests that the unconjugated bilirubin fraction is likely responsible for the overall increase in the total serum bilirubin, an indication of hepatic cells dysfunction.
The results obtained from the biochemical assays, indicating conditions of liver damage, were supported by the prominent histopathological changes observed to be associated with exposure to nitrocellulose thinner in this present study (Figures 2-5). The histopathological results showed that administration of nitrocellulose thinner, particularly at the concentrations of 15 and 20 mg/kg body weight, for one month resulted in damage of liver structure along with disarrangement of hepatic strands. Several hepatic cells were observed to show different histological features of necrosis, ranging from diffuse cytoplasmic vacuolation (ballooning degeneration), to pyknotic nuclei of hepatocytes with lymphocytic infiltration of the hepatic portal triad. Moreover, an enlargement of the sinusoids dilation and congestion of blood vessels with hemorrhage were very prominent in the liver of rats exposed to 20 mg/kg body weight of nitrocellulose thinner (Figure 5).

5. Discussion

The present study assessed the effect of oral administration of nitrocellulose thinner, at varying concentrations, on the liver function capacities in male rats. The results of this investigation indicate that oral administration of nitrocellulose thinner to rats caused significant alterations in both biochemical parameters and liver tissue histopathological status at the different test concentrations; although the groups of rats administered 15 and 20 mg/kg weight of nitrocellulose thinner were observed to be the more affected. The activities of serum ALT, AST, GGT and ALP, and the levels of conjugated and total bilirubin in the serum, were observed to be significantly increased, while the values of total protein and total albumin were statistically decreased in all the test animals. However, higher percentage increase in the activities of serum ALT and GGT than AST and ALP, serum levels of total than conjugated bilirubin, and percentage decrease in albumin than total serum protein, were recorded in this study. Also, severe histopathological changes, such as centrilobular hepatic necrosis, fatty change, kupffer cell, ballooning degeneration, and infiltrating lymphocytes were observed to be associated with exposure to nitrocellulose thinner.

It is generally known that liver function tests may be conducted through blood assays to give information about the state of the liver, describing its synthetic functionality (using albumin), cellular integrity (using transaminases, particularly ALT), and its link with the biliary tract (using GGT and ALP). ALT is the enzyme produced within the cells of the liver, recording increases in conditions where liver cells have been inflamed or undergone cell death. Generally, liver enzymes such as ALT, AST and ALP are marker enzymes for liver function and integrity (Jens and Hanne, 2002; Adaramoye et al., 2008). These enzymes are usually raised in acute hepatotoxicity or mild hepatocellular injury, but tend to decrease with prolonged intoxication due to damage to the liver (Cornelius, 1979; Jens and Hanne, 2002). As the cells are damaged, the ALT leaks into the bloodstream leading to a rise in the serum levels. It is the most sensitive marker for liver cell damage. Histopathological changes are also very important in the assessment of the functional state of the liver tissues (Uboh et al., 2010; Edem, 2009). On the basis of the foregoing, the results of this study give a strong indication that exposure to nitrocellulose thinner at concentrations higher than 10 mg/kg may be a predisposing factor for liver tissue damage, and hence dysfunctions.

The results of this present study are in agreement with the results of the different previous researches which indicated that the exposure to different chemical agents led to induce severe physiological and biochemical disturbances of the liver tissues in experimental animals (Adaramoye et al., 2008; Adedapo et al., 2004; Jens and Hanne, 2002). Particularly, Tos-Luty et al., (2003) showed that malathion intoxication led to severe effects on the structures of the liver and kidney including the presence of fine subcapsular infiltrations.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>GGT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.87 ± 2.88</td>
<td>14.83 ± 3.44</td>
<td>24.32 ± 4.22</td>
<td>260.38 ± 20.48</td>
</tr>
<tr>
<td>10 mg/kg of NCT</td>
<td>20.04 ± 4.02*,+</td>
<td>23.65 ± 4.08*</td>
<td>48.73 ± 3.56*</td>
<td>350.53 ± 28.34*</td>
</tr>
<tr>
<td>15 mg/kg of NCT</td>
<td>28.26 ± 3.78*,+</td>
<td>30.04 ± 5.221,+</td>
<td>68.86 ± 5.421,+</td>
<td>378.62 ± 26.48*,+</td>
</tr>
<tr>
<td>20 mg/kg of NCT</td>
<td>30.08 ± 5.25*,+</td>
<td>33.24 ± 4.86*+,+</td>
<td>72.88 ± 6.22*,+</td>
<td>390.06 ± 22.56*,+</td>
</tr>
</tbody>
</table>

All values are presented as mean ± SEM, n=6, *p<0.05 compared with the Control, +p<0.05 compared with 10 mg/kg of NCT; NC=Nitrocellulose thinner.
Table 2: Effect of Nitrocellulose thinner on some serum liver function assessment metabolites

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Albumin (mg/dl)</th>
<th>Total Protein (mg/dl)</th>
<th>Conj. Bili. (mg/dl)</th>
<th>Total Bili. (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.24 ± 2.06</td>
<td>6.62 ± 1.84</td>
<td>1.65 ± 0.46</td>
<td>1.96 ± 0.62</td>
</tr>
<tr>
<td>10 mg/kg of NCT</td>
<td>3.40 ± 1.32</td>
<td>5.02 ± 1.34</td>
<td>2.20 ± 1.31</td>
<td>3.42 ± 1.22*</td>
</tr>
<tr>
<td>15 mg/kg of NCT</td>
<td>2.20 ± 0.42*</td>
<td>3.40 ± 1.56*</td>
<td>2.84 ± 1.04*</td>
<td>5.85 ± 2.04*</td>
</tr>
<tr>
<td>20 mg/kg of NCT</td>
<td>2.02 ± 0.52*</td>
<td>3.01 ± 1.04*</td>
<td>3.10 ± 1.22*</td>
<td>6.02 ± 1.48*</td>
</tr>
</tbody>
</table>

All values are presented as mean ± SEM, n=6, *p<0.05 compared with the Control. NCT=Nitrocellulose thinner, Conj=Conjugated, Bili.=Bilirubin.

diffused parenchymatous degeneration of single hepatocytes, and the presence of fine foci constructed of plasmatic cells and histiocytes located between hepatic plates. The present study showed a significant increase only in the levels of ALT, a state which can be associated with cell necrosis of many tissues (Adedapo et al., 2004). The data made available from the results of this study suggest that the composite constituents of nitrocellulose thinner exert possible hepatotoxic effect in rat model. This is evidenced by the increase in the activities of serum ALT, AST, GGT and ALP activities, conjugated and total bilirubin levels, as well as decrease in albumin and total protein; indicators of liver tissue damages. However, the basic mechanism through which nitrocellulose thinner exerts this toxicity effect is still not clearly understood. But it is generally known that all types of liver inflammation, including cell damage, can cause elevations of these liver enzymes in the serum due to leakage. The level of increase of these enzymes in the serum is known to correlates with the number of cells damaged (Fleming, 2006). Based on the observations made from the results of this present study, it may be suggested that the constituents of nitrocellulose thinner, or their metabolites, are reactive and might have interacted with the liver tissues and caused damages to the tissues. It is therefore believed that these damages, on one hand, caused the enzymes to leak and increased the serum activities, as seen by greater increase in serum ALT and GGT activities, with a relatively lower increase in serum AST and ALP activities; while on the other hand, adversely altered the functional state of the liver tissues, as indicated by a higher percentage increase in total bilirubin above the conjugated fraction, and higher percentage decrease in serum albumin above total serum protein. In support of the results obtained from this study, high serum ALT levels have also been reported by Lundberg et al. (1994) among the painters, who possibly are frequently exposed to nitrocellulose thinner in the course of their occupational activities. The results of this study are also in correlation with the results of the study of Patil et al. (2007), on the effect of occupational lead exposure in battery manufacturing workers, silver jewellery workers and spray painters on liver functions. Moreover, Dioka et al., (2005) and Uboh et al., (2007, 2008, 2009), reported similarly that additives and other composite constituents of petrol and petroleum solvents, which are also likely to be present in nitrocellulose thinner, have adverse health effects in both human and experimental animals. Also, Boogaard et al., (2005) observed and reported biochemical alterations in renal and hepatic functions of operators employed in a chemical plant producing chlorinated hydrocarbons.

![Image of a bar graph showing the effect of nitrocellulose thinner on serum liver function assessment metabolites](image-url)
**Figure 1b:** Effect of nitrocellulose thinner on comparative percentage changes in some serum liver function assessment metabolites. Total serum protein (TSP), Conjugated bilirubin (Conj. Bili.), Total bilirubin (TB)

![Bar chart showing percentage changes in liver function metabolites](chart)

**Figure 2:** A section of a normal liver tissue from rats in the Control group, showing the central vein and normal hepatocyte

![Normal liver tissue](image1)

**Figure 3:** A section of liver tissue of rats administered 10mg of nitrocellulose thinner, showing inflamed portal triad and hepatocytes

![Liver tissue with inflammation](image2)

**Figure 4:** A section of liver tissue from rats administered 15mg nitrocellulose thinner, showing mild diffuse cytoplasmic vacuolation (ballooning degeneration), pyknotic nuclei of hepatocytes with lymphocytic infiltration of the hepatic portal triad

![Liver tissue with mild degeneration](image3)

**Figure 5:** A section of liver tissue from rats administered 20mg nitrocellulose thinner, showing severe diffuse cytoplasmic vacuolation (ballooning degeneration), pyknotic nuclei of hepatocytes with lymphocytic infiltration of the hepatic portal triad

![Liver tissue with severe degeneration](image4)
References


