**Assessment of Hepatic and Oxidative Stress Biomarkers Alterations in workers Exposed to Automobile Body Paint Spraying at Angwan Rogo, Jos-North LGA, Plateau State.**

**Abstract**

This study aims at ascertaining the effects of prolonged exposure to automobile spray paint on liver function enzyme and non-enzyme biomarkers, as well as oxidative stress biomarkers in automobile body painters. Sixty five (65) healthy human volunteer subjects of ages 18-55 years were recruited and grouped into five according to their duration in the profession: Group 1 (Control), Group 2 (1-6 years), Group 3 (7-12 years), Group 4 (13-18 years) and Group 5 (19 years and above). An informed consent form was administered to them to seek their approval for participation in the study. A semi-structured questionnaire was also administered to them in order to collect their bio-data and their knowledge of automobile spray painting. Both liver function and oxidative stress biomarkers were determined using spectrophotometric method. The findings reveal a significant increase (p < 0.05) in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels, particularly in workers with longer exposure durations, indicating potential hepatocellular injury. Additionally, variations in total protein (TP), albumin (ALB), and bilirubin levels suggest systemic metabolic disturbances. Furthermore, oxidative stress biomarkers exhibit notable alterations, including a significant reduction in superoxide dismutase (SOD) and glutathione (GSH) levels, alongside an elevation in malondialdehyde (MDA), implicating oxidative stress as a key mechanism of toxicity. These biochemical changes corroborate prior research, reinforcing concerns regarding occupational exposure to toxic compounds in automotive paints. The study underscores the urgent need for enhanced workplace safety measures, including adequate ventilation, protective equipment, and routine health assessments for spray painters. Future research should explore long-term health consequences and potential mitigation strategies to reduce occupational hazards in this industry.

**Keywords:** Liver biomarkers, bilirubin, automobile spray painting, oxidative stress.

1. **INTRODUCTION**

Workers around the world are facing a global health crisis due to occupational exposure to toxic chemicals. Every year, more than one billion workers are exposed to hazardous substances, including pollutants, dusts, vapour and fumes in their working environment. Globally, over 2,780,000 workers die as a result of unsafe work condition and ethics per year [1]. United Nations’ report published in 2018, by [2], the United Nations’ special rapporteur on toxics, stated that a worker dies in every 30 seconds as a result of exposure to toxins and in every 15 seconds, a worker dies from dangerous working conditions in general. Studies have shown that many workers are exposed to toxicants in their work environment in Nigeria, thereby, making them susceptible and vulnerable to some adverse health conditions.

However, automobile spray painting is one of such occupations that expose workers to toxins. It exposes the workers to chemicals and also produces high concentration of dust during the process of scraping (removing) the old paint before applying (spraying) the new one. This technique involves the use of abrasive sanders to smoothen the metal surface before spraying new paint and it produces abrasive of silica, rust along with cadmium and lead that come from sanding coat-painting surface [3]*.* In most places, particularly in the study area, visit to auto-painting sites has shown that quite a large number of automobile body sprayers do not appreciate the importance of safety equipment and protective clothing. Therefore, the workers can easily inhale the chemical constituents of the paints, aerosols and respirable dust particles generated during scraping of the old paint because most of the work areas do not have proper ventilation. The situation worsens where the workers are not aware of the health risk associated with exposure to those chemicals and the toxins it introduces into their work environment. Most oil base paints used on vehicles contain some heavy metals such as lead, cadmium, mercury and chromium, despite the availability of other pigments that can be used as their substitutes. Additionally, studies have shown that accumulation of heavy metals in the body is dangerous and can result to kidney damage, miscarriage, learning and memory difficulties [4].

Additionally, major sources of these heavy metals include paint pigments and additives which have been overlooked in the past. Study has indicated that children are the most vulnerable of all classes to suffer neurological disorder after ingestion of small amount of these heavy metals contained in paint [5]. Serious health effects of lead poisoning have elicited global campaign to eliminate lead use in paint. The major challenge is the dearth of data on lead levels in paints from developing countries. Lead levels of paints manufactured and sold in Nigerian market were studied [6].

In developed nations like the United Kingdom, auto-painting is a task that requires the skill and expertise of professionals, taking necessary safety and precautionary measures with all kinds of personal protective equipment [7]. However, in developing countries like Nigeria, particularly in Jos, a visit to some auto-painting sites has proven that most of the workers do not take all the necessary precautionary measures and do not make use of personal protective equipment (PPE). Therefore, this study aims at assessing some of the occupational health risk faced by automobile body painters due to inhalation and skin exposure to heavy metals, solvents as well as other components used in automobile-spray painting.

In addition, several researches have been carried out on the assessment of heavy metal contents of some oil-based paints produced both locally and internationally. There might be limited or few existing research, specifically focusing on the occupational health risks associated with those involved in automobile body painting in the Jos metropolis. This research gap necessitates a dedicated study to fill the knowledge void and provide insights into the specific challenges faced by workers in this locality.

1. **MATERIALS AND METHODS**
   1. **Ethical Approval**

The ethical approval for this study was obtained from the Research Ethics Committee of Plateau State Specialist Hospital, Jos, Plateau State, Nigeria and was assigned Reg. No: NHREC/05/01/2010b.

* 1. **Sample size**

Snow-ball sampling technique was adopted for the study and the sample size was determined using Atchley’s formula [8].

n = N

1 + *N* (e)2

Where:

* *n* is the sample size
* *N* is the population size
* *e* is the margin of error
  1. **Exclusion criteria**

Subjects excluded from participating in this study are non-automobile painters or other artisans that do not engage in painting activities from the study area. Workers below the age of 18 and above 55 years were excluded from the study. Individuals with known medical condition were not recruited.

* 1. **Inclusion criteria**

Subjects included are automobile spray painters who gave their consent to participate in the study within the study area. Active and healthy who have been in the profession for at least one years and those who fall within the ages of 18-55 years were included in the study.

* 1. **Experimental Design**

Sixty five (65) healthy human volunteer subjects of ages 18-55 years were recruited and grouped into five (Group 1, Group 2, Group 3, Group 4 and Group 5) at Angwan Rogo, Jos-North LGA, Plateau State, Nigeria. An informed consent form was administered to them to seek their approval for participation in the study. A semi-structured questionnaire was also administered to them in order to collect their bio-data and their knowledge of automobile spray painting.

Group 1 (control) comprises those who are not involved in automobile spray painting.

Group 2 comprises those who have been in automobile spray painting for 1-6 years.

Group 3 comprises those who have been in automobile spray painting for 7-12 years.

Group 4 comprises those who have been in automobile spray painting for 13-18 years.

Group 5 comprises those who have been in automobile spray painting for 19 and above years.

* 1. **Collection of Samples**

Five (5) ml of blood sample was collected into non-EDTA (plain) vacutainer closed sample tubes with the help of a professional. The samples were spun in a centrifuge at 4,000 rpm for 5 minutes and the serum was harvested using pasteur pipette into micro-vials for onward analyses in the laboratory.

* 1. **Analysis of Liver Function Biomarkers**

Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT)were determined by the method of [9]. Alkaline Phosphatase (ALP) was determined by method of [10]. Total protein (TP) was estimated using the biuret method. Total and Direct bilirubin were determined using spectrophotometric method as described by [11]. Albumin was determined using Bromocresol Green (BCG) Dye-Binding Assay method as described by [12].

* 1. **Analysis of Oxidative Stress Biomarkers**

Superoxide dismutase (SOD) activity was determined by the method described by [13]. The method of [14] was followed to determine the level of glutathione. Catalase activity was determined using the method described by [15]. Malondialdehyde (MDA) was determined according to method described by [16].

1. **RESULTS**
   1. Socio-demographic Characteristics of the Respondents(Automobile Painters).

Table 1 shows the socio-demographic characteristics of the respondents. A total of fifty two questionnaires were distributed, filled and returned. All the 52 (100%) respondents were male automobile spray painters. Majority of them are were married 36 (69%), 10 (19%) were singles, 2 (4%) were divorced, 1 (2%) was widowed, 3 (6%) were cohabiting. The age ranges of the respondents: 18-27 years were 17 (33%), 28-37 were 12 (23%), 38-47 were 19 (36%), 48 and above years were 4 (8%). Out of the 52 respondents, 34 (65%) had secondary education, 11 (21%) of the respondents had primary education, 4 (8%) had tertiary education, 3 (6%) had no form of education.

**Table 1: Socio-demographic Characteristics of Respondents**

|  |  |  |
| --- | --- | --- |
| **Variable** | **Frequency** | **Percentage (%)** |
| **Types of Respondents** |  |  |
| Automobile Painters | 52 (52) | 100 |
| Control | 13 (13) | 100 |
| **Total** | 65 |  |
| **Gender of Respondents** |  |  |
| Automobile Painters |  |  |
| Male | 52 | 100 |
| Female | 0 | 0 |
| **Total** | 52 |  |
| **Marital Status** |  |  |
| Automobile Painters |  |  |
| Married | 36 (52) | 69 |
| Single | 10 (52) | 19 |
| Divorced | 2 (52) | 4 |
| Widowed | 1 (52) | 2 |
| Cohabiting | 3 (52) | 6 |
| **Total** | 52 | 100 |
| **Age of Automobile Painters (Years)** |  |  |
| 18-27 | 17 (52) | 33 |
| 28-37 | 12 (52) | 23 |
| 38-47 | 19 (52) | 36 |
| 48 and above | 4 (52) | 8 |
| **Total** | 52 | 100 |
| **Education Status of Painters** |  |  |
| None | 3 (52) | 6 |
| Primary | 11 (52) | 21 |
| Secondary | 34 (52) | 65 |
| Tertiary | 4 (52) | 8 |
| **Total** | 52 | 100 |

* 1. **Respondents’ Knowledge of the Constituents of Paints and its other Components.**

Table 2 shows respondents’ knowledge of the constituents of paint. Majority of the respondents, 41(79 %) were not aware of the constituents of paints while 11(21 %) of the respondents were aware.

**Table 2: Knowledge of Constituents of Paints and Other Components by Painters.**

|  |  |  |
| --- | --- | --- |
| **Knowledge of Painters** | **Frequency** | **Percentage (%)** |
| Yes | 11 (52) | 21 |
| No | 41 (52) | 79 |
| **Total** | 52 | 100 |

* 1. **Painters Awareness of Health Hazards Associated with the Use of Paint.**

Table 3 shows the respondents knowledge of hazards associated with the use of pain. Majority of the respondents, 39 (75 %) were not aware of health hazards associated with the use of paint and its other components, while 13 (25 %) were aware of hazards associated with the profession.

**Table 3: Awareness of Hazards associated with Automobile Spray Painting by Respondents**

|  |  |  |
| --- | --- | --- |
| **Awareness of Painters** | **Frequency** | **Percentage (%)** |
| Yes | 13 (52) | 25 |
| No | 39 (52) | 75 |
| **Total** | 52 | 100 |

* 1. **Use of Personal Protective Equipment (PPE) among Painters.**

Table 4 shows the personal protective equipment and their frequency of usage by the respondents. The results show that a larger percentage of the respondents 35 (67%), do not use these safety clothing. It has also been observed that only 2(4%) use overalls, 7 (13%) use nose mask, 1 (2%) use goggles, 2 (4%) use gloves, while 5 (10%) use shoes.

**Table 4: Use of Personal Protective Equipment (PPE) by Respondents.**

|  |  |  |
| --- | --- | --- |
| **Personal Protective Equipment (PPE)** | **Frequency** | **Percentage (%)** |
| If yes, what type of PPE? |  |  |
| 1. Overall | 2 (52) | 4 |
| 1. Nose mask | 7 (52) | 13 |
| 1. Goggles | 1 (52) | 2 |
| 1. Gloves | 2 (52) | 4 |
| 1. Shoes | 5 (52) | 10 |
| **Total** | 17 | 33 |

* 1. **Reported Symptoms among Painters.**

Table 5 shows the common adverse health symptoms reported by the respondents. It has been observed that 15 (29%) reported symptoms relating to hazard exposure, 37 (71%) reported that there were no symptoms. Difficulty in breathing was the most frequently reported adverse health effect 7(13%). Others include irritation to the skin 4(8%), cough 2(4%) and 2(4%) reported multiple symptoms.

**Table 5: Reported Symptoms among Respondents (Automobile Spray Painters)**

|  |  |  |
| --- | --- | --- |
| **Symptoms** | **Frequency** | **Percentage (%)** |
| Skin irritation | 4 (52) | 8 |
| Cough | 2 (52) | 4 |
| Breathing difficulty | 7 (52) | 13 |
| Multiple symptoms | 2 (52) | 4 |
| **Total** | 15 | 29 |

* 1. **Effects of duration variation on liver function enzyme biomarkers of automobile body painters.**

Table 6 shows the effects of duration variation on liver function enzyme biomarkers of automobile body painters. This result reveals significant increase in AST in group 4 (76.62+4.445) and group 5 (74.00+2.815) p < 0.05, when compared with the control group (58.39+3.131). The present study shows an elevated ALT in group 5 (23.00+0.506) p < 0.05, when compared with the control group (18.15+0.905). The ALP value has also been observed to be significantly high in group 4 (40.89+2.324) p < 0.05, when compared with the control group (30.17+2.258).

**Table 6: Effects of duration variation on liver function enzyme biomarkers of automobile body painters**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S/N** | **Duration** | **AST( μ/l)** | **ALT( μ/l)** | **ALP( μ/l)** |
| 1 | Group 1 | 58.39+3.131 | 18.15+0.905 | 30.17+2.258 |
| 2 | Group 2 | 60.54+4.006c | 18.39+0.866c | 33.07+2.684c |
| 3 | Group 3 | 63.62+3.016c | 19.77+0.968c | 34.50+2.235c |
| 4 | Group 4 | 76.62+4.445a | 21.00+0.555c | 40.89+2.324a |
| 5 | Group 5 | 74.00+2.815a | 23.00+0.506a | 39.62+2.684c |
|  | P-Value | 0.0003 | 0.0002 | 0.0121 |

Values are expressed as mean ± SEM, n = 13.

aValues on the same column are significantly different when compared with control (p < 0.05).

cValues on the same column are not significantly different when compared with control (p ˃ 0.05).

**AST=**Aspartate amino transferase, **ALT=**Alanine amino transferase, **ALP=**Alkaline phosphatase.

* 1. **Effects of duration variation on liver function non-enzyme biomarkers of automobile body painters.**

Table 7, shows the effects of duration variation on liver function non-enzyme biomarkers of automobile body painters. The TP has been observed to be significantly higher in group 2 (75.54+1.226), group 3 (76.32+1.935) and group 5 (75.71+2.230) p < 0.05, when compared with the control group (67.13+1.212). The present study shows significant higher value of ALB only in group 5 (34.75+0.321) p < 0.05, when compared with the control group (32.28+0.251). The result of this study shows a significantly lower value of TB in group 3 (00.36+0.024) p < 0.05, when compared with the control group (00.55+0.041). Lower values of DB were observed in group 3 (00.28+0.019) and group 4 (00.26+0.018) p < 0.05, when compared with the control group (00.38+0.220).

**Table 7: Effects of duration variation on liver function non-enzyme biomarkers of automobile body painters**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S/N** | **Duration** | **TP(g/l)** | **ALB(g/l)** | **TB(mg/dl)** | **DB(mg/dl)** |
| 1 | Group 1 | 67.13+1.212 | 32.28+0.251 | 00.55+0.041 | 00.38+0.220 |
| 2 | Group 2 | 75.54+1.226a | 32.89+0.228c | 00.43+0.033c | 00.36+0.023c |
| 3 | Group 3 | 76.32+1.935a | 32.43+0.272c | 00.36+0.024a | 00.28+0.019a |
| 4 | Group 4 | 73.50+1.968c | 33.47+0.419c | 00.45+0.032c | 00.26+0.018a |
| 5 | Group 5 | 75.71+2.230a | 34.75+0.321a | 00.65+0.039c | 00.38+0.019c |
|  | P-Value | 0.0026 | 0.0001 | 0.0001 | 0.0001 |

Values are expressed as mean ± SEM, n = 13.

aValues on the same column are significantly different when compared with control (p < 0.05).

cValues on the same column are not significantly different when compared with control (p ˃ 0.05).

**TP**= Total protein, **ALB**=Albumin,**TB=**Total bilirubin, **DB=**Direct bilirubin.

* 1. **Effects of duration variation on oxidative stress biomarkers of automobile body painters.**

Table 8 shows result of the effects of duration variation on oxidative stress biomarkers of automobile body painters. The SOD has been observed to decrease significantly in group 4 (17.82+0.357) and group 5 (18.16+0.668) p < 0.05, when compared with the control group (24.43+1.262). This study reports significant decrease in GSH in group 4 (07.75+0.282) and group 5 (07.48+0.272) p < 0.05, when compared with the control group (09.25+0.346). As observed from the result of this study, a significantly higher CAT has been recorded in group 3 (11.25+0.745), group 4 (10.52+0.778) and group 5 (10.06+0.685) p < 0.05, when compared with the control group (14.00+0.608). MDA has shown elevated values in group 4 (02.01+0.148) and group 5 (02.13+0.108) p < 0.05, when compared with the control group (01.12+0.060).

**Table 8: Effects of duration variation on oxidative stress biomarkers of automobile body painters.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S/N** | **Duration** | **SOD**  **(µmol SOD/min/mg protein)** | **GSH**  **(µg/ml)** | **CAT**  **(µmoleH2O2/min/mg Protein)** | **MDA**  **(mmol/mg Protein)** |
| 1 | Group 1 | 24.43+1.262 | 09.25+0.346 | 14.00+0.608 | 01.12+0.060 |
| 2 | Group 2 | 24.18+1.425c | 09.12+0.292c | 13.23+0.593c | 01.52+0.137c |
| 3 | Group 3 | 21.49+1.120c | 08.92+0.318c | 11.25+0.745a | 01.50+0.109c |
| 4 | Group 4 | 17.82+0.357a | 07.75+0.282a | 10.52+0.778a | 02.01+0.148a |
| 5 | Group 5 | 18.16+0.668a | 07.48+0.272a | 10.06+0.685a | 02.13+0.108a |
|  | P-Value | 0.0001 | 0.0013 | 0.0003 | 0.0001 |

Values are expressed as mean ± SEM, n = 13.

aValues on the same column are significantly different when compared with control (p < 0.05).

cValues on the same column are not significantly different when compared with control (p ˃ 0.05).

**SOD**=Superoxide-dismutase, **GSH**=Glutathione, **CAT**=Catalase, **MDA**=Malondialdehyde.

1. **DISCUSSION**

Table 6 shows the effects of duration variation on liver function enzyme biomarkers of automobile body painters. This result reveals significant increase in AST in group 4 (76.62+4.445) and group 5 (74.00+2.815) p < 0.05, when compared with the control group (58.39+3.131). However, increase in AST concentrations were observed in automobile body spray painters as duration of exposure increases. Aspartate aminotransferase (AST) is a crucial enzyme involved in amino acid metabolism. It is primarily present in the liver but can also be found in red blood cells, the heart, muscle tissue, the pancreas and the kidneys. AST levels play a significant role in diagnosing liver diseases, though they are not specific to any particular condition. However, when measured alongside other enzymes, AST can help determine the underlying cause of various liver disorders. Elevated AST activity may also be associated with myocardial infarction [17. This is in conformity with the findings of [18] and [19], which also reported that AST increases in proportion to the duration of workers in automobile spray painting. The liver metabolizes toxic organic solvents, which have the potential to damage its membranes. This may explain the increased levels of Aspartate Aminotransferase observed with prolonged exposure to car paint over the years. A related study by [20], also highlights the physiochemical harm to body organs caused by reactive metabolites from paint. The researchers linked this damage to the high lipophilicity of these metabolites, which can develop after prolonged exposure to car paint.The present study shows an elevated ALT in group 5 (23.00+0.506) p < 0.05, when compared with the control group (18.15+0.905). This trend is an indication that automobile spray painting impacts more on the body system with a concomitant increase in duration of workers in the profession. Prolonged exposure to toxic substances can impact organs like the liver, which is responsible for metabolizing these harmful compounds. As a result, liver enzyme production may be affected. Alanine aminotransferase (ALT) plays a crucial role in converting alanine, an amino acid present in liver and kidney cells. Measuring ALT levels in the blood can help assess liver function and identify potential causes of liver disorders. An ALT test is often used to track the progression of liver conditions such as hepatitis or liver failure. When toxic solvents enter the body, they are processed by the liver, potentially leading to liver damage and disruptions in enzyme production [21]. The present study agrees with the finding of [20], which also reported elevated ALT but contradicts the study of [22], which reported reduced ALT. The ALP value has also been observed to be significantly high in group 4 (40.89+2.324) p < 0.05, when compared with the control group (30.17+2.258). This outcome agrees with the findings of [23], though refutes the report of [24], which reported non-significant difference in liver function biomarkers of automobile body spray painters. The elevation of these liver function enzyme biomarkers in this study could be due to hepatocellular injury which leads to the release of the enzymes into the blood stream. This could be attributed to exposure of workers to heavy metals and toxic organic compounds used in the production of automobile spray paints and other components used in spray painting.

Table 7 shows the effects of duration variation on liver function non-enzyme biomarkers of automobile body painters. The TP has been observed to be significantly higher in group 2 (75.54+1.226), group 3 (76.32+1.935) and group 5 (75.71+2.230) p < 0.05, when compared with the control group (67.13+1.212). Proteins serve as essential components of all cells and tissues, playing a crucial role in body growth, development, and overall health. They contribute to the structure of various organs and are key elements in the formation of enzymes and hormones that regulate bodily functions. This study contrasts the report of [25]. The present study shows significant higher value of ALB only in group 5 (34.75+0.321) p < 0.05, when compared with the control group (32.28+0.251). This outcome contradicts report by [25], which stated that Total protein and Albumin levels are rarely raised except in dehydration and artefactually by prolonged venous stasis. The result of this study shows a significantly lower value of TB in group 3 (00.36+0.024) p < 0.05, when compared with the control group (00.55+0.041). Lower values of DB were observed in group 3 (00.28+0.019) and group 4 (00.26+0.018) p < 0.05, when compared with the control group (00.38+0.220).

Table 8 shows result of the effects of duration variation on oxidative stress biomarkers of automobile body painters. The SOD has been observed to decrease significantly in group 4 (17.82+0.357) and group 5 (18.16+0.668) p < 0.05, when compared with the control group (24.43+1.262). This finding agrees with the report of [26], which also reported reduced SOD in artisans exposed to heavy metals. This study reports significant decrease in GSH in group 4 (07.75+0.282) and group 5 (07.48+0.272) p < 0.05, when compared with the control group (09.25+0.346). The present study is in concordance with the findings of [27], which reported decrease in GSH in auto paint workers. As observed from the result of this study, a significantly higher CAT has been recorded in group 3 (11.25+0.745), group 4 (10.52+0.778) and group 5 (10.06+0.685) p < 0.05, when compared with the control group (14.00+0.608). This result refutes the finding of [23], which reported increased level of CAT in auto painters. On the other hand, MDA has shown elevated values in group 4 (02.01+0.148) and group 5 (02.13+0.108) p < 0.05, when compared with the control group (01.12+0.060). This study is in agreement with the report of [28], which also reported elevated level of MDA in exposed subjects. Elevated SOD, GSH, CAT and the reduced MDA could be attributed to heavy and other toxicants in the paint and other components which might have induced oxidative stress via lipid peroxidation [23].

1. **CONCLUSION**

The findings of this study indicate that prolonged exposure to automobile spray paint significantly affects liver function enzyme and non-enzyme biomarkers, as well as oxidative stress biomarkers in automobile body painters. The observed increase in AST, ALT, and ALP levels suggests possible hepatocellular injury due to toxic solvent exposure, leading to the release of these enzymes into circulation. Additionally, variations in total protein, albumin, and bilirubin levels further highlight the systemic impact of prolonged exposure to hazardous compounds present in automotive paints.

Furthermore, the study reveals a decline in SOD and GSH levels, alongside an increase in MDA, signifying oxidative stress likely caused by heavy metals and other toxicants in paint formulations. These biochemical alterations corroborate previous findings, reinforcing the notion that occupational exposure to spray paints poses a substantial risk to liver health and overall physiological balance.

The results underscore the need for improved occupational safety measures, including proper ventilation, use of protective equipment, and regular health monitoring for workers in the automobile spray painting industry. Future research should focus on long-term health implications and potential interventions to mitigate the adverse effects of exposure.

**COMPETING INTEREST**

Authors have declared that no competing interest exist.

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