

Original Article

GLUE ABUSE IN LOME: INVESTIGATION AND TOXICOLOGICAL STUDY ON WISTAR RATS

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ABSTRACT

Objectives: Volatile substances abuse (VSA) is considered as one of the most dangerous forms of drug abuse leading to serious accidents and fatalities. This study aims to assess the extent and dangers of the sniffing of "Dia" glue, used by the vulcanizer to paste the cars' tires.

Methods: The first phase of the study involved a survey with vulcanizers of the district I of Lome. The effect of the glue was then evaluated on female wistar rats, by inhalation' in a 1l capacity jar for a period of 5 min at doses of 320 mg/l and 640 mg/l. The rat behavioral changes, driving test, tail flick test, tolerance test and 28 d subchronic toxicity test were carried out.

Results: The survey has identified street vendors as glue sniffers (80.95%). The most cited reason for the glue inhalation was the tailism (79.76%) and the presumed effect was to feel stronger (76.19%). On wistar rat, "Dia" glue has induced some behavioral changes. It has increased significantly the righting recovery reflex time and the maintaining time of the tail in warm water. After 28 d exposition, 5 min per day, "Dia" glue increased significantly ($p < 0.001$) the relative weight of the spleen, the AST ($p < 0.001$) and ALT ($p < 0.001$). It has also induced an anaemia associated with a thrombocytosis. The analysis of the glue by GC-MS has showed a high amount of toluene (65%), a lesser proportion of dimethyl cyclohexane, ethyl acetate and traces of benzene, ethylbenzene and xylene.

Conclusion: The sniffing of "Dia" glue is very dangerous. Addiction especially that which is done with volatile substances must, therefore, be carefully controlled.

Keywords: Glue sniffing, Sensorimotor function, Subchronic test, Toxicological analysis, Toluene

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INTRODUCTION

Dependence and intentional abuse of volatiles as alternative drugs have been reported since antiquity¹. In addition, this plague was reported in many countries, South Africa [1]; Israel [2]; Canada [3] and France [4]. Volatile substance abuse is one of the most dangerous forms of drug abuse, more pernicious, the most widespread and most toxic which can lead to serious accidents [5, 6]. Volatile substances abuse (VSA) can cause serious damage to the integrity of the central nervous system and the neurobiological development [7, 8]. Recent reports from Egypt [9], Israel [8], Slovakia [10] and USA [11] highlight health problems caused by these substances.

On the list of the most commonly products containing volatiles substances used in this type of drug abuse, we have glue, nail polish, and the petrol lighter [12]. The prevalence of this pernicious form of addiction is partly due to their easy accessibility unlike conventional drugs (cannabis, cocaine, heroin and crack) [7]. The easy accessibility to volatile substances by young people allows them to abuse freely since they don't need much money to buy.

VSA is unfortunately also present in Togo. Some people sniff glue, we will call in this study "Dia" glue, produced in Taiwan and used by vulcanizers to paste cars' inner tubes tires in case of a puncture. We aim, therefore, in this study, to assess the extent and dangers of the sniffing of "Dia" glue.

MATERIALS AND METHODS

The study was conducted following an approved animal use protocol from the institutional Ethical Committee for Teaching and Research (ref no. CNCB-CEER 2801/2015). Animal care and handling are conducted as conformed to accepted guidelines [13, 14]

Animals

Female Wistar rat (150-200 g), provided by the department of Animal Physiology of University of Lome (Togo) were used. They were housed in a standard environmental condition and fed with standard rodent diets and water ad libitum.

Survey

A cross-sectional survey in a single pass was used in this study. It has been taken place from 09th to 21st June, 2014. The study was conducted with vulcanizers of the district I of Lome. One hundred vulcanizers have participated in the study. The study was done using a simple random sampling method. We have included in the study vulcanizers and their apprentices present at our passage and having freely accepted to participate to this survey.

Vulcanizers and their apprentices who had not given their consent were not included. For this study, we used a semi-structured questionnaire around the main items of information about the glue sniffing. The interviews were conducted in the workplace of vulcanizers. All have been done by the same investigator. The entry, verification and analysis of data were made through software Microsoft Excel 2007.

Toxicological study

In order to have an idea on the toxicity of "Dia" glue a preliminary study on some parameters have been done such as the effect of the glue on sensorimotor rat function and the 28 d subchronic toxicity test.

Study of rat sensorimotor function

To evaluate the rat sensorimotor function, the animal behavioral changes, the righting recovery reflex time, the tail flick test and a tolerance test have been used. To eliminate the diurnal change, all tests were done at 09:00–12:00 A. M.

For each of the pre-cited test, 24 female rats were used; they were divided into three groups of 8 animals each. Group 1 was not exposed to the glue. Group 2 and 3 were exposed to the glue respectively at the doses of 320 mg/l and 640 mg/l, by inhalation in a 1L capacity jar during a period of 5 min. Doses of 320 mg/l and 640 mg/l were chosen on the basis of the dose inhaled by sniffers. Mean sniffer weight (60 g) used a tube of 2 g per day which corresponds to 320 mg/l.

The righting recovery reflex (RRR) time was used first. Rats were placed in the supine position in order to record the time to recover the righting reflex. This period is defined as the time rats have made to the return to upright position, with all four paws oriented towards the ground [3]. The tail-flick test was used to assess the thermal nociceptive threshold. During the tail-flick test, the tail was immersed in a warm bath (55 ± 0.5 °C) until rat withdrawal it (flicking response). This test was used because some sniffers have said that the make it in order to stay without pain.

The development of tolerance to glue (320 and 640 mg/l/kg) was evaluated by the tail flick test after 5 min exposition to the glue per day for 28 d.

Subchronic toxicity test

The repeat oral dose toxicity's study was carried out according to OECD guideline 408 [13]. Female rats were divided into three groups of 8 animals each. Group 1 was not exposed to the glue. Group 2 and 3 were exposed to the glue respectively at the doses of 320 mg/l and 640 mg/l, by inhalation in a 1L capacity jar during a period of 5 min. Animals have been exposed daily for 28 d at a similar time. Animals were observed at least twice daily for morbidity and mortality. Body weight of animals was evaluated daily.

On the 29th day, after an overnight fast, rats were anaesthetized with ether and blood sample for haematological and biochemical analysis were collected into tubes with or without EDTA respectively. Haemoglobin (Hb), haematocrit (Ht), red blood cells count (RBC), white blood cells count (WBC), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and platelet count were determined using automatic counter Sysmex (K21, Tokyo, Japan). Biochemical analysis were performed in serum obtained after centrifugation of total blood without anticoagulant, at 2500 rpm for 15 min Standardized diagnostic kits (Labkit®) and a Biotron® spectrophotometer were used for spectrophotometrical determination of the following biochemical parameters: alanine aminotransferase (ALT), aspartate amino-transferase (AST), creatinine, alkaline phosphatase (ALP), glucose (Glu), total proteins, γ GT and urea. Necropsy of all animals was carried, and the organ weights (heart, liver, kidney and spleen) were recorded. Each weighed organ was then standardized for percentage body weight of each rat (relative organ weight). Histological study of organs was done after sacrificing the animals on 29th day.

Toxicological analysis

The glue analyzes were done by gas chromatography (GC-MS Shimadzu QP2010) coupled to mass spectrometry with injection space head (Perkin Elmer Headspace Turbo Matrix HS, HS-GC-MS). These analyzes was performed at University Hospital in Limoges (France). The GCHS-MS has been performed according to the following criteria: the Column used is a 624 Rxi Sil MS 0.25 mm internal diameter and 30 meters in length, the injector temperature is 110 °C.

Statistical analysis

The results are expressed as mean \pm standard error of the mean (SEM). Statistical analysis was performed by one-way analyse of variance (ANOVA) with Tukey test to evaluated significant differences between groups. Values of $p < 0.05$ were considered significant. All statistical analysis was carried out using the In-stat Statistical package (Graph Pad software, Inc. USA).

RESULTS

One hundred vulcanizers, with a mean age of 37.3 y, have participated in the survey. Around 84.52% of them have said that they were eyewitness of glue sniffing, and two of them have

accepted that the sniff "Dia" glue. The glue tube cost 2 \$ (91%). Street vendors were identified as glue sniffers (80.95%). The most cited reasons for the inhalation are tailism (79.76%), curiosity (51.19) and pleasure (25%). The presumed effects for that are to feel stronger (76.19%) to escape or to relax (42.86%) and stay without pain (10.71%).

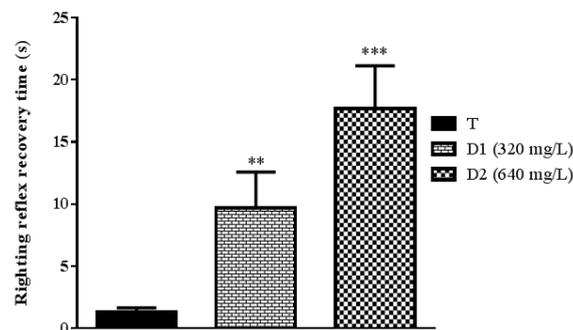


Fig. 1: Effect of the "Dia" glue on the righting reflex recovery time. Rats were placed in the supine position in order to record the time of the recovery of righting reflex (RRR). This period is defined as the time rats have made to the return to upright position, with all four paws oriented towards the ground. Asterisk indicates significantly different from the controls (ANOVA and Tukey test, $p < 0.05$)

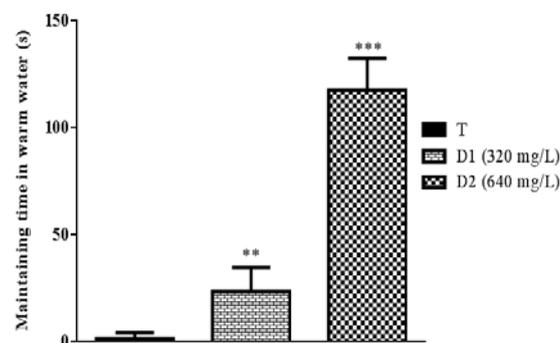


Fig. 2: Effect of the "Dia" glue on the maintaining time of the tail in a warm water. The tail was immersed in a warm bath (55 ± 0.5 °C) until tail withdrawal (flicking response). Asterisk indicates significantly different from the controls (ANOVA and Turkey test, $p < 0.05$)

About the animal behavioral evaluation, animals in the control group (T) and the group D1 (320 mg/l) have showed no toxicity signs. Animals of group D2 have showed a tremor of forelimbs from the 2nd min accompanied by ataxia, adynamia, piloerection followed by drowsiness that appeared between 3rd and 4th min After the exposure, behavioral changes have disappeared in seconds that followed.

"Dia" glue has at, 320 mg/l and 640 mg/l, increased significantly (** $p < 0.01$) the righting recovery reflex time (fig. 1) and the maintaining time of the tail in warm water (fig. 2) as compared to the control. After a single exposure to the glue, the maintaining time has been 1.03 \pm 0.03s, 23.41 \pm 11.12 and 117.7 \pm 14.59s respectively for the control, the group D2 and the group D3 (table 1). During 28 d of exposure, the glue has caused a significant decrease in the duration of maintenance of tail in the water. For example on the 28th day, the control group maintaining time is closed to that of the first day. But for the treated rat (320 mg/l and 640 mg/l) the maintaining time was significantly less than that on the first day (23.41 \pm 11.12s and 117.7 \pm 14.59s respectively for the group D2 and D3) (table 1).

During the subchronic study, "Dia" glue has not caused any significant changes in rats weight body (table III). At 640 mg/l "Dia" glue has significantly ($p < 0.01$) increased the relative weight of the

spleen, the AST, and the ALT (table 3). At the same doses, "Dia" glue increased significantly the platelet rate ($*P<0.001$) but has significantly decreased red blood cells ($**P<0.01$), white blood cells ($*P<0.05$) and hemoglobin ($***P<0.001$) (table 4). No significant damage was observed in rat liver, kidney, spleen, lung and testis

tissues of treated rat at the histological evaluation. The HS-GC-MS analysis has shown that "Dia" glue is composed of 96% of liquid solvent mainly toluene (at least 65% of mass) (data was not shown) and a lesser proportion of dimethyl cyclohexane and ethyl acetate. We have noted traces of benzene, ethyl benzene and xylene.

Table 1: Effect of glue on maintaining of the tail time in warm water during 28th days of exposure

Days	Control	Glue "Dia"	
		320 mg/l	640 mg/l
1	1.03±0.03	9.69±2.89***	117.7±14.59***
7	1.59±0.23	4.31±0.99**	65.02±23.62***
14	1.88±0.12	3.03±0.76*	5.15±0.82****
21	1.29±0.35	2.29±0.63*	4.95±1.21****
28	1.12±0.22	2.87±0.13	4.56±0.85****

The results are mean±SEM with N=8; *P<0.05 (control group versus extract); **P<0.01 (control group versus extract); ***P<0.001 (control group versus extract); ****P<0.001 (control group versus extract); *****P<0.001 (control group versus extract).

Table 2: Mean body weight (g) of rats after 28 d treatment with the "Dia" glue

Week	Control	Glue "Dia"	
		320 mg/l	640 mg/l
0	156±5.51	148±2.27	149±7.01
1	155±5.66	149±1.78	152±6.70
2	154±3.51	145±2.39	146±6.72
3	156±5.36	148±4.02	144±6.20
4	151±3.33	147±2.39	148±7.01

The results are mean±SEM with N=8

Table 3: Rat organ relative weight (%) after 28 d treatment with "Dia" glue

Week	Control	Glue "Dia"	
		320 mg/l	640 mg/l
Heart	0.37±0.01	0.40±0.02	0.35±0.03
Liver	3.34±0.27	2.97±0.16	2.92±0.24
Spleen	0.16±0.01	0.17±0.01	0.20±0.01**
Lungs	0.49±0.03	0.54±0.03	0.51±0.03
Kidney	0.64±0.03	0.65±0.04	0.61±0.05

The results are mean±SEM with N=8; **P<0.01 (control group versus extract)

Table 4: Rats biochemical parameters after 28 d treatment with "Dia" glue

Week	Control	"Dia" glue	
		320 mg/l	640 mg/l
ASAT (U/l)	97±1.22	114.6±8.3**	115.5±5.19***
ALAT (U/l)	41.5±5.69	42.83±2.14	55.83±6.62*
PAL (U/l)	95.3±32.92	177.1±30.50	127.8±23.27
Urea (g/l)	0.33±0.05	0.30±0.07	0.30±0.07
Blood sugar (mg/dl)	1.13±0.09	1.08±0.11	1.24±0.16
Creatinine (mg/dl)	5.83±0.65	5.12±0.31	5±0.52

The results are mean±SEM with N=8; *P<0.05 (control group versus extract); **P<0.01 (control group versus extract); ***P<0.001 (control group versus extract)

Table 5: Rat Haematological parameters for rats after 28 d treatment with "Dia" glue

Parameter	Control	Glue "Dia"	
		320 mg/l	640 mg/l
WBC (10 ³ /μl)	10.03±1.53	8.88±0.16	7.57±1.63*
RBC (10 ⁶ /μl)	8.03±0.08	7.61±0.06**	7.49±0.18**
Haemoglobin (g/dl)	15.96±0.56	14.33±0.19***	13.68±0.22***
Haematocrit (%)	43.7±0.89	42.9±0.50	42.66±0.92
MCV (fl)	55.15±0.73	55.26±0.49	53.36±0.92
MCH (pg)	18.52±0.39	18.4±0.17	18.08±0.18
MCHC (%)	33.7±0.38	33.37±0.18	32.76±0.25
Platelet (10 ³ /μl)	393.8±39.59	416.2±14.77 **	442.4±13.82***

The results are mean±SEM with N=8; *P<0.05 (control group versus extract); **P<0.01 (control group versus extract); ***P<0.001 (control group versus extract).

DISCUSSION

The volatile substances abuse is considered as one of the most dangerous forms of drug abuse. In this study, we have shown the psychoactive effect of the "Dia" glue by evaluating the animal behavioral changes, righting recovery time, the tail flick time and the tolerance test. The exposition to the "Dia" glue at 320 mg/l and 640 mg/l has induced a significant increase in the righting reflex recovery time. This result has demonstrated the decrease of motor activity in rats treated. This motor activity decrease, associated with ataxia and somnolence confirm the CNS depressant effect of "Dia" glue. It is well known that a substance that calms animals is a sedative, a CNS depressant, a muscle relaxant, or a paralyzing anesthetic [15]. Several studies have noted the CNS depressant effect due to volatile solvents (including glues) [16, 17]. "Dia" glue at 320 mg/l and 640 mg/l has increased significantly ($p < 0.05$; $P < 0.001$) the nociceptive threshold after exposure. The withdrawal of the tail is a spinal response [18]. This analgesic effect developed by "Dia" glue in this work confirms its action on the CNS. After 28 consecutive days of exposure to the glue, there was a significant decrease in the nociceptive threshold. This decrease in the nociceptive threshold has shown that after some day's exposure, the same doses do not cause the same effects on animals and that we must increase the dose to get the effect previously observed. Then "Dia" glue has induced tolerance in animals. Therefore, people who abuse in the glue-sniffing would risk developing a tolerance, which means that a larger quantity would be needed by sniffers in order to have the desired effects. The excessive and prolonged consumption of volatile substances is not without danger to the user according to the literature [19]. To confirm dangers of prolonged use of "Dia" glue, we have studied the 28 d subchronic toxicity of "Dia" glue.

At the end of the subchronic test, "Dia" glue has increased significantly ($p < 0.01$) the relative weight of the spleen of rats. The evaluation of the weight of organs such as the liver, kidney, spleen, testes, heart, pancreas, brain and lung is very important in toxicological studies. The weight of a body or even more, the relative weight is an important indicator used in physiology and toxicology [20]. Toxicology and Pharmacology Society (STP) recommends organ relative weight determination for toxicity studies in between one week to one year [21]. But it is difficult to associate the spleen relative weight increase' to the "Dia" glue because of the wide variation in the spleen and other lymphoid organs from one animal to another [22].

"Dia" glue has also decreased some haematological parameters (WBC, RBC, Hb) and increased platelet counts after 28 d giving normocytic normochromic anemia. The hematopoietic system is one of the favorite target of toxic substances, and consequently, an important parameter of the physiopathology of human or animal [23]. The increase' in the relative weight of the spleen caused by "Dia" glue (640 mg/kg) can be related to anemia observed in treated rats. Indeed, haemolysis is generally associated to spleen weight increase' due to an accumulation of iron in the body and sometimes in spleen cells [24]. The HS-GC-MS analysis has shown that "Dia" glue is composed of 96% of liquid solvent mainly toluene (at least 65 % of mass) and a lesser proportion of dimethyl cyclohexane and ethyl acetate. We note traces of benzene, ethyl benzene and xylene. Since 2006, toluene is classified in the category 3 of carcinogenic, mutagenic and reprotoxic substances. The implementation of market glue and spray paints intended for public sale and containing more than 0.1% of toluene was forbidden in Europe [25]. Toluene is known to induce dizziness, depression and fatigue in paint workers [26]. Acute poisoning with ethyl acetate causes a headache, dizziness and CNS depression. As for dimethyl cyclohexane, it mainly causes CNS depression [27]. Therefore, the CNS depression we have obtained during our study can be due to these volatiles substances present in this glue. Anaemia, thrombocytosis and hepatic impairment we have observed at the end of 28 d of exposure to "Dia" glue may be due to the presence of dimethyl cyclohexane which is known to be toxic to blood and liver [27].

The presence of traces of ethyl benzene, xylene and especially of benzene in "Dia" glue is very dangerous effects. Benzene, a haematotoxicity and an suppressive immune chemical, is classed in the category 1 of carcinogenic chemicals by IARC [28].

Glue sniffing is an extremely dangerous practice. Indeed, by its composition, "Dia" glue may cause CNS depressant, carcinogenic, mutagenic, reprotoxic, hepatotoxicity, hematopoietic, making her a real poison.

CONFLICT OF INTERESTS

Declared none

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