Hematological and biochemical effects of an air freshener in rabbits

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Abstract: The present investigation deals with the effect of inhalation of the air freshener, glade on hematological and biochemical parameters in male rabbits. Animals were divided into 3 groups. Animals of first group were considered as controls and animals of second anf third groups, were inhaled one ml of the air freshener, Glade, once daily for 2 and 4 weeks respectively. Animals were kept individually in a closed cage and 1 ml of the air freshener was sprayed in each cage. Exposure of animals to glade for 2 and 4 weeks induced significant decrease in RBCs count, hemoglobin, WBCs count hematocrit percentage and the blood platelets. Transaminases ALT and AST were gradually increased in the sera of treated rabbits and this increase became significant ($P<0.05$) at the end of the fourth week.
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1. Introduction

Air pollutants found indoors are of particular concern since on average people spend 85-90% of their time indoors (USEPA, 1996). Many household products are sources of volatile organic compounds, an important class of air pollutants that are typically found in concentrations several fold higher indoors when compared to outdoors (Wallace, 1991). Spiller (2004) investigates the toxic effect of volatile substance for 6-year period (1996-2001). He reported that three categories were responsible for the majority of deaths: gasoline (45%), air fresheners (26%), and propane/butane (11%). Anderson and Anderson (1997) reported that air freshener cause sensory, pulmonary irritations and decreases in airflow velocity in addition to abnormalities of behaviour measured by the functional observational battery score. On the other hand, Marsolek, *et al*. (2010) reported that Butane, propane, and air fresheners had the highest fatality rates among children aged 12 to 17 years. Air fresheners have become a staple in many homes and offices, marketed with the promise of creating a clean, healthy, and sweet-smelling indoor atmosphere. But many of these products contain hazardous chemicals (ref.). Hydrocarbon compounds are present in many household products. Wickramasinghe et al. (2012) reported that polycyclic aromatic hydrocarbons, one of persistent organic pollutants, in the atmosphere may be a major etiological factor in lung carcinogenicity. However, dichloromethane inhalation cause severe damage in the central nervous system, including the optic nerve (Kobayashi et al., 2008). Zhang *et al*. (2011) found that 1,2-dichloroethane inhalation could induce toxic encephalopathy with a pathological feature of cerebral edema. Recreational abuse of hydrocarbons by inhalation is accomplished in 3 ways: sniffing, huffing, and bagging (ref). Sniffing, the least potent delivery method is the inhalation of the volatile substance through the nostrils (i.e, sniffing glue). Tsao et al. (2011) reported that toluene, aromatic hydrocarbon, sniffing can lead to cardiac arrhythmias and sudden sniffing death syndrome. Also, Hogie *et al*. (2009) reported that toluene inhalation induces visual stimulation; the eye velocity was slower and more irregular than in the control state. He added that at the end of the stimulation, the environment of the animals became stationary, but the eye did not immediately return to a fixed stable position. Huffing is the placing of a rag soaked with an inhalant such as gasoline or lighter fluid over the nose and mouth. Bagging involves repeated deep inhalations from a plastic or paper bag filled with a particular hydrocarbon such as spray paint or another propellant (Lorenc, 2003). Swiercz *et al.* (2011) reported that inhalation of 2-methylnaphthalene vapours for 4 weeks at different concentration increasing γ-glutamylotransferase activity, stimulation of the hematopoietic system, lower cholesterol concentrations, higher number of goblet cells in lobar bronchi and hyperplasia of hepatic bile ducts. Uzun and Kendirli (2005) reported that patients with neurologic symptoms who abused toluene as an inhalant, nearly one third showed deficits in orientation, attention, learning, arithmetic calculation, abstraction, construction, and recall. Glade is an air freshener widely used in many countries. On the other hand, it showed some hazardous effect. LoVecchio *et al*. (2001) reported a case of a patient who survived ventricular fibrillation after inhalation of Glade air freshener, which contains short chain aliphatic hydrocarbons (butane and isobutane). The present work studied the effect of inhaling glade on hematological and biochemical features of rabbits.

2. Materials and Methods

Animals and treatment

Male New Zealand white rabbits weighing 1.8-2 kg were housed in the laboratory at controlled light and

temperature. They provided with rabbit chow and fresh water. Animals were divided into 3 groups:

Group 1. Animals of this group (5 rabbits) were considered as controls.

Group 2. Animals of this group (15 rabbits) each inhaled one ml of the air freshener, Glade, once daily for 2 weeks.

Group 3. Animals of this group (15 rabbits) each inhaled one ml of the air freshener, Glade, once daily for 4 weeks. Animals were kept individually in a closed cage and 1 ml of the air freshener was sprayed in each cage. Glade is obtained from local markets, Makkah, Saudi Arabia.

Hematological and biochemical assays

For hematological study, blood was collected from control and treated animals after 2 and 4 weeks of treatment. The hematological parameters: red blood cells count (RBCS), hemoglobin value (Hb), hematocrit value (HCT %), white blood cells count (WBCS) and blood platelets number were measured by a fully automated Coulter counter (Coulter Electronics Limited, England). For biochemical study sera were obtained by centrifugation of the blood Samples and stored at 20°C until assayed for the biochemical parameters. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using a fully automated Hitachi 911 analyzer (Tokyo, Japan). A commercial randox kits (Randox Laboratories, LTD, Ardomre, Crumlin, United Kingdom) were used in these analysis.

Statistical analysis:

The results are given as mean ± standard deviation (X± S.D.). Significance of the differences was tested by the Student "t" test. The levels of significance were taken at $p < 0.05$.

3. Results and Discussion

Exposure to air refreshing sprays/atomisers is consistent with the known susceptibility of asthmatic subjects to odours, which have been related to respiratory complaints, including tightness of the chest, shortness of breath, wheeze and cough (Baldwin *et al*., 1999). In addition, cleaning products and cologne/perfume are among the most common triggers reported by asthmatics reacting to odours (Shim and Williams, 1986). Exposing rabbits to the air freshener, glade, in the present work caused significant decrease in the number of RBCs', WBC's, hemoglobinhematocrit and platelets number (table 1). Glade contains hydrocarbons, namely butane and isobutene, The hematological effects of hydrocarbon components were studied by several investigators. Chou *et al*. (2010) found a significant differences in weekly mean hemoglobin, red blood cell count, and hematocrit (HCT) between exposed soldiers and controls following exposure to hexachloroethane-zinc oxide. Okoro *et al*. (2000) reported that inhalation of hydrocarbons fumes causes depression of total white blood cell count as well as red blood cell count and its dependent haematological indices (PCV, Hb), MCH and MCV. Prolonged exposure to certain aromatic hydrocarbons (especially benzene), can lead to an increased risk of aplastic anemia, multiple myeloma, and acute myelogenous leukemia (Cody et al.1993). Frequent exposures to gasoline vapours or any of the hazardous constituents (particularly benzene, hexane, tetraethyl lead and xylene) are reported to be haematotoxic (d'Azevedo et al.1996).

White blood cells function primarily in body defense against foreign bodies and this is often achieved through leucocytosis and antibody production. In this study, the white blood cell count decreased significantly in rabbits inhaled glade. The decrease in WBC observed in this study is possibly as a result of pancytopaenia and leukocytopaenia, which may result in impaired migration of phagocytic cells, lower resistance to viruses, bacteria and foreign bodies (Marieb,1995). This observation is similar to that recorded by Ovuru and Ekweozor (2004) in rabbits exposed to crude oil hydrocarbons.

Significant increase in liver function enzymes, ALT and AST, was recorded in animals exposed to glade(Figs.1&2). This result is in agreement with McIntyre and Long (1992) who reported liver function impairment in a 17 year old abuser of butane aerosols. A total of 30-45% of an inhaled dose of butane is absorbed and though most is excreted unchanged via the lungs a small amount is probably metabolized in the liver (Flanagan et al.1996). Sotaniemi et al.(1982) reported that chronic low-dose industrial exposure to related chlorinated hydrocarbons and benzol derivatives causes mild abnormalities of serum aminotransferases. It was reported that elevations in serum levels of these enzymes were mostly attributed to acute hepatocellular damage or extrahepatic obstruction, or both (Sherlock,1981).

Toxicity of inhalation of hydrocarbon compounds were reported by some investigators. Sugie et al. (2004) reported three cases of sudden death due to inhalation of portable cooking stove fuel (case 1), cigarette lighter fuel (case 2), and liquefied petroleum gas (case 3). n-Butane was the major substance among the volatiles found in the tissues of cases 1 and 2, and propane was the major substance in case 3. A combination of the autopsy findings and the gas analysis results revealed that the cause of death was ventricular fibrillation induced by hard muscle exercise after gas inhalation in cases 1 and 2, and that the cause of death in case 3 might be hypoxia. Fuke et al.(2002) reported that n-Butane has an anesthetic or narcotic effect on the central nervous system, and induce fatal arrhythmia. The authors added that n-butane substances are lipophilic so that after being taken up from the lungs into blood, they distributed at high concentrations in lipid-rich tissues such as brain and fat tissues, and also

in liver, heart, and kidney. Inhalation of butane caused an increase in oxygen demand, coronary artery spasm, platelet aggregation, and thrombus formation (El-Menyar et al.2005). The used air freshener, glade contains butane and isobutene. It is suggested that the hematological and biochemical effects recorded in the present work may be resulted as a toxic effect of butane and/or isobutene.

Table 1. Effect of glade inhalation on blood parameters of male rabbits

Treatment Parameters	Group 1 "Control"	Group 2 "2 weeks"	Group 3 "4 weeks"
RBCs $10^6/mm^3$	4.62±0.6	4.17±0.4	2.52±0.7*
Hb gm/dl	11.5±1.2	9.2±0.3	7.9±0.2*
HCT %	28.5±1.1	24.6±1.5	18.1±1.3*
Platelets $10^3/L$	412±1.2	334±2.4	110±33*
WBCs$10^6/mm^3$	5.7±0.3	3.6±0.7*	3.3±0.4*

-Values are expressed as mean ± SD - (*) Significant at $p<0.05$

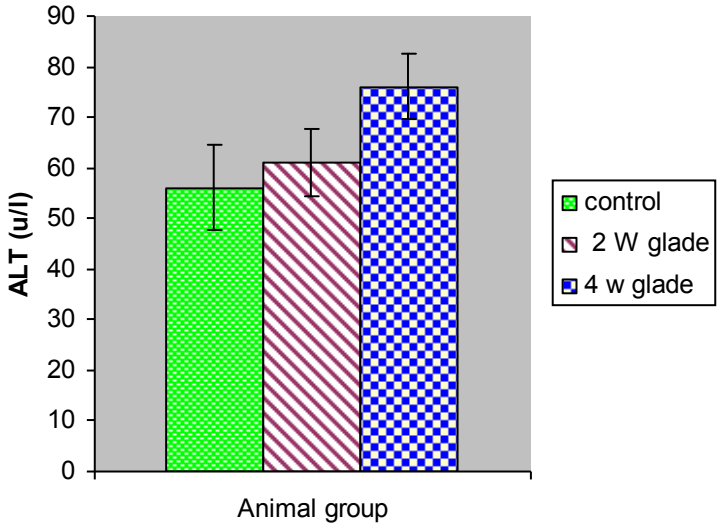

Fig.1.Effect of glade inhalation on ALT

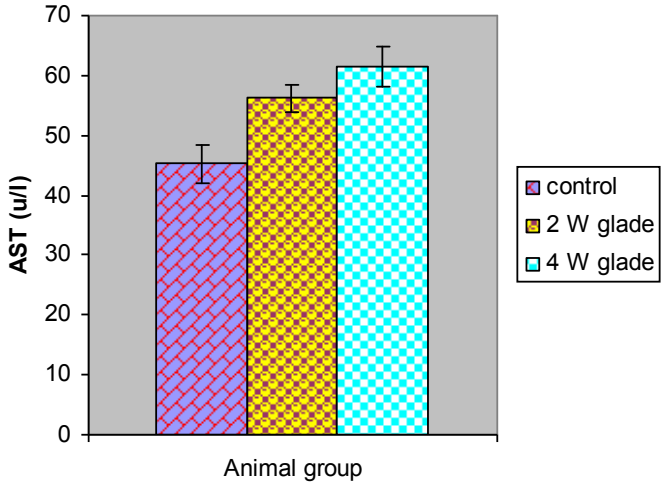

Fig.2. Effect of glade inhalation on AST

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