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RESEARCH ARTICLE

The Effects of Liquid Dishwashing Detergent Exposure in Male Swiss Albino Mice

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ABSRACT

The current study investigated the possible effects of liquid dishwashing detergent on some blood parameters and organ weights of male Swiss Albino mice. A total of 40 male Swiss albino mice were used in the experiment. Mice were randomly assigned to 5 treatment groups (n=8 per group). Mice in the control group (TR1) were supplied with tap water. Meanwhile, mice in TR2, TR3, TR4 and TR5 groups were given 0.1, 0.5, 1 and 5% v/v of the liquid detergent in tap water, respectively. The treatments started at 3 wk of age and continued for 60 d. Mice were kept under standard laboratory conditions in separate plastic cages. Food and water were provided ad libitum. At the end of the 60 d treatment period, blood samples were collected from the beating hearth under anesthesia. After sacrificed, the organs of the mice were weighted. There were no significant alterations on hematocrit, RBC, WBC, MCV, MCH, MCHC, total lymphocyte, monocyte or basophil levels ($P>0.1$) due to detergent exposure. Similarly, treatments had no effect on liver, kidney, spleen, lung, testis or brain weights ($P>0.1$). However, ingestion of detergent adversely affected hemoglobin ($P < 0.01$), plasma protein ($P<0.05$), total neutrophil ($P<0.03$) and eosinophil ($P<0.03$) levels. Furthermore, ALP and AST levels were significantly increased in TR4 and TR5 ($P<0.05$) due to detergent exposure. Thus, the results of the current study imply that particularly the higher doses of liquid dishwashing detergent could be toxic and cause health risks to male Swiss albino mice.

Keywords: detergent, toxicity, hematology, liver enzymes

Swiss Albino Erkek Farelerde Sıvı Bulaşık Deterjanı Maruziyetinin Etkilerinin İncelenmesi

ÖZ

Bu çalışmanın amacı sıvı bulaşık deterjanının erkek Swiss Albino farelerinde kan parametreleri ve organ ağırlıkları üzerine etkisinin araştırılmasıdır. Bu amaçla 40 sağlıklı Swiss albino fare rastgele olarak, her grupta 8 fare olacak şekilde, toplam 5 gruba ayrıldı. Kontrol grubundaki farelere (TR1) içme suyu olarak sadece musluk suyu verilirken, TR2, TR3, TR4 ve TR5 grubunda bulunan farelere su kaynağı olarak sırasıyla sadece 0.1, 0.5, 1 ve 5% sıvı deterjan ihtiva eden musluk suyu verildi. Çalışma fareler 3 haftalıkken başladı ve 60 gün süresince devam etti. Fareler standard laboratuvar koşulları altında plastik kafeslerde muhafaza edildi. Besin ve su ad libitum olarak sağlandı. Uygulamanın sonunda farelerden Sevorane anestezisi altında kan örnekleri toplandı. Kan örnekleri toplandıktan sonra hayvanlar öldürülerek iç organları çıkarıldı ve tartıldı. Bu çalışmanın sonucuna göre uygulanan deterjan dozlarının hematokrit, alyuvar, akyuvar, ortalama alyuvar hacmi, ortalama alyuvar hemoglobini, ortalama alyuvar hemoglobin yoğunluğu, total lenfosit, monosit ve bazofil değerleri üzerine herhangi bir etkisine rastlanmadı ($P>0.1$). Ayrıca, deterjan uygulamasının karaciğer, böbrek, dalak, akciğer, testis ve beyin ağırlıkları üzerinde etkisinin olmadığı belirlendi ($P>0.1$). Ancak, hemoglobin($P<0.01$), plazma protein ($P<0.05$), total nötrofil ($P<0.03$) ve eozinofil ($P<0.03$) seviyelerinin deterjan uygulamasından olumsuz etkilendiği gözlendi. Bununla birlikte, ALP ve AST seviyeleri TR4 ve TR5 gruplarında önemli bir şekilde yüksek bulundu ($P<0.05$). Bu çalışmadan elde edilen veriler ışığında, özellikle yüksek dozlarda deterjan uygulamasının erkek Swiss albino farelerde toksik etki yapabileceği ve sağlık problemlerine yol açabileceği sonucuna varıldı.

Anahtar kelimeler: deterjan, toksisite, hematoloji, karaciğer enzimleri

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INTRODUCTION

Detergents are designed for cleaning purposes. They are often used for assisting the removal of dirt or other materials from contaminated surfaces. The active chemical ingredients of detergents are termed as surfactants or surface-active agents (Scott and Jones, 2000; Yahaya et al. 2011). Thus, they are called as amphiphilic substances because one part of the detergent is hydrophilic and the other part is hydrophobic. In general practice, classic anionic "detergent" refers to alkylbenzenesulfonates. The alkylbenzene part of these detergents is lipophilic and the sulfonate part is hydrophilic (Prats et al. 1997).

Two varieties of detergents have been popular: branched alkyl groups and linear alkyl groups. Today, mostly detergents with branched alkyl groups are in use since detergents with linear alkyl groups are poorly biodegradable. The total quantity of linear alkylbenzene sulphonates (LAS) were around 1million ton/year in the United States, Japan and Western Europe (Ritchler and Knaut, 1991). The surfactant production in Western Europe was estimated to be around 3 million tons in 2010 (CESIO-Stats 2010).

Detergents are commonly used in two major markets, household detergents and industrial/institutional cleaning products. In addition, they are being used for pesticide formulations. However, household detergents are the major source of chemical substances of domestic origin which are discharged into the environment. Both industrial and domestic premises consume more than 1.2 million tons surfactants in Europe (Morse 1999). As a result, the increasing domestic health hazards and environmental pollution are arising from detergent exposure (Warne and Schifko, 1999).

The detergent exposure can pose serious health risks to human and animals. However, the effects of detergent exposure on mammalian health are poorly studied. Thus, the aim of the current study was to investigate the toxicity of liquid dishwashing detergent on some blood parameters and organ weights of male Swiss Albino mice.

MATERIALS and METHODS

Animals and Diets

The study was approved by the ethics committee of Suleyman Demirel University (08/12/2009.28.05) Total of 40 healthy male Swiss albino mice were used. The mice were kept in plastic cages, under standard laboratory conditions (12 h light/dark and 22^{o}C). Pelletized commercial feeds were given ad libitum (Korkuteli Yem, Antalya, Turkiye; 88% dry matter, 8%ash, 23% crude protein, 5% crude fiber). All treatments started right after weaning (3-weeks of age) and continued for 60 days.

Experiment and Sampling

A commonly used dish washing liquid was purchased from local market and used in the current experiment. The chemical constituents of the detergent included anionic and nonionic surfactants, ethanol and ethanolamine. Initial weights of the mice were taken and the animals randomly assigned into 5 different treatment groups (n=8 per group). The control mice (TR1) were resigned in cage 1 and received tap water. The treated mice in cages 2, 3, 4 and 5 were given 0.1 (TR2), 0.5 (TR3), 1 (TR4) and 5 (TR5) % v/v of the liquid detergent solutions in tap water, respectively as the only source of water. Water intake was checked and noted daily in all treatment groups.

At the end of the experiment, mice were kept under sevorane anesthesia and body weights were recorded. Each mouse was cut through the chest region in a dorsal-ventral direction and blood samples were collected into Na-heparinized capillary tube from the beating hearth. After blood collection, animals were sacrificed and internal organs were removed. The weights of the organs were recorded.

Blood Parameters

Plasma protein levels were measured by using hand-held refractometer (Atago, SPR-N, Japan) at 22°C by the same person. Hemoglobin was measured spectrophotometrically by using the cyanmethemoglobin method (Bhaskaram et al. 2003). Microhematocrit method was used for the hematocrit measurements (Chernecky and Berger, 2001).

For counting red blood cell (RBC), the blood was diluted 200 times with the Hayem solution. For counting white blood cells (WBC), the blood was diluted 10 times with the Turck's solution. Subsequently, dilutions were counted in the Thoma counter compartment at 40X magnification (Nageswari and Kothari, 2007).

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentrations (MCHC) were calculated by the help of total erythrocytes, hematocrit, and hemoglobin values (Chernecky and Berger, 2001). Percent leukocyte distribution were determined from the blood smears that were stained with May Grünwald–Giemsa stain.

Alkaline phosphatase (ALP) and aspartate aminotransferase (AST) concentrations were measured by auto analyzer (Gesan Chem 200).

Statistical Analyses

Results are presented as mean ± SE. Data were analyzed by one way ANOVA and Tukey post hoc tests. All statistical analyses were carried out using SAS statistical package. The minimum level of significance was set at $p<0.05$.

RESULTS

The mice in the current study showed no clinical signs of toxicity. The water consumption among the groups did not differ (TR1 = 5.61± 1.29, TR2 = 6.27 ± 1.15, TR3 = 6.44 ± 1.46, TR4 = 6.17± 1.59 and TR5 = 6.31 ± 1.40 ml/day/mouse, $P >0.1$). No changes in hematocrit, RBC, WBC, MCV, MCH, MCHC, total lymphocytes, monocytes, or basophils were apparent among the treatments ($P > 0.1$; Tables 1 and 2). Moreover, the weights of liver, kidney, spleen, lung, testis and brain were not altered due to detergent exposure ($P > 0.1$; Table 3). On the other hand, the results of the hematologic examination indicated a marked reduction on total hemoglobin ($P < 0.01$) and plasma protein ($P < 0.05$) levels due to detergent treatment (Tables 1 and 2). Detergent treatments also resulted in increased total neutrophil ($P < 0.03$) and total eosinophil ($P < 0.03$) levels. In addition, serum ALP and AST levels were significantly elevated in TR4 and TR5 ($P < 0.05$; Tables 1 and 2).

Table 1. Effect of liquid dishwashing detergent on plasma protein levels, and some hematological and biochemical parameters of Swiss Albino mice after oral gavages for 60 days.

	TR1	TR2	TR3	TR4	TR5	P<
Plasma Protein (g/dL)	6.88±0.26^{a}	6.52±0.13^{a}	6.71±0.27^{a}	5.98±0.11^{b}	6.08±0.31^{b}	0.05
RBC (x10^{6})	9.56±0.72	8.73±0.29	8.66±0.26	8.50±0.19	8.32±0.25	NS
Hemoglobin (g/dL)	15.2±0.39^{a}	14.7±0.35^{ab}	14.4±0.56^{bc}	12.7±0.54^{c}	13.1±0.40^{c}	0.01
Hematocrit (%)	39.4±1.23	38.1±2.28	37.1±1.82	35.0±1.68	34.75±2.16	NS
MCV (fL)	42.4±2.23	43.3±2.43	43.9±3.03	41.2±3.21	42.0±2,42	NS
MCH(pg)	16.5±1.08	16.9±0.68	16.7±1.10	15.0±0.81	15.7±0.51	NS
MCHC(g/dL)	38.8±1.82	39.6±2.45	39.2±2.04	37.1±2.02	38.2±2.05	NS
ALP (U/I)	42.7±1.82^{a}	43.4±2.74^{a}	44.3±2.25^{a}	50.6±2.92^{b}	51.4±2.75^{b}	0.05
AST (U/I)	24.9±0.56^{a}	24.7±0.61^{a}	25.6±0.75^{a}	27.5±0.81^{b}	28.4±0.73^{b}	0.05

a,b,c: Averages in groups in the same row with different superscripts are statistically important; NS= Not significant.

Table 2. Effect of liquid dishwashing detergent on white blood cells (WBC) of Swiss Albino mice after oral gavages for 60 days.

	TR1	TR2	TR3	TR4	TR5	P <
WBC (x10^{3})	5.06±0.43	5.13±0.56	5.38±0.23	6.06±0.31	7.33±1.62	NS
Lymphocytes (x10^{3}/μL)	3.91±0.37	3.92±0.53	4.14±0.17	4.36±0.34	5.05±1.19	NS
Neutrophils (x10^{3}/μL)	0.83±0.08^{a}	0.78±0.13^{a}	0.91±0.05^{a}	1.04±0.10^{b}	1.68±0.41^{b}	0.03
Monocytes (x10^{3}/μL)	0.23±0.05	0.22±0.07	0.31±0.05	0.32±0.04	0.37±0.03	NS
Eosinophils (x10^{3}/μL)	0.071±0.01^{a}	0.096±0.02^{a}	0.082±0.03^{a}	0.272±0.09^{b}	0.263±0.08^{b}	0.03
Basophils (x10^{3}/μL)	0.008±0.004	0.011±0.007	0.013±0.008	0.014±0.009	0.016±0.007	NS

a,b: Averages in groups in the same row with different superscripts are statistically important; NS=Not significant.

Table 3. Effect of liquid dishwashing detergent on body and some organ weights of Swiss Albino mice after oral gavages for 60 days.

	TR1	TR2	TR3	TR4	TR5	P<
Body Weight (g)	30.5±0.51	30.3±0.52	29.1±0.42	30.3±0.50	29.2±0.41	NS
Liver (g)	1.39±0.06	1.47±0.05	1.37±0.06	1.40±0.05	1.37±0.06	NS
Right Kidney (g)	0.22±0.01	0.22±0.01	0.21±0.02	0.23±0.02	0.22±0.01	NS
Spleen (g)	0.10±0.006	0.09±0.005	0.09±0.005	0.10±0.006	0.10±0.005	NS
Lung (g)	0.20±0.01	0.20±0.01	0.19±0.02	0.18±0.01	0.18±0.01	NS
Brain (g)	0.37±0.01	0.38±0.02	0.35±0.02	0.36±0.01	0.38±0.01	NS

NS=Not significant.

DISCUSSION

The marked changes in some of the blood parameters of detergent treated mice are supported by the previous studies. The alterations in haematological profiles due to a surfactant, sodium dodecyl sulfate, in rabbits (Wadaan and Mubarak, 2009) and an anionic detergent in Sprague Dawley rats (Dehelean et al. 2004) had been previously reported. Haematological profiles are known to provide important information about the internal environment of mammals. The environmental factors reported to pose a variety of adverse effects on the haematological profiles of most organisms. In the current study, there was a decrease in hemoglobin levels of the mice in TR4 and TR5. It is possible that the constituents in detergent might have interacted with hemoglobin synthesis. Decrease in hemoglobin synthesis could be associated with liver damage, or iron absorption from small intestine.

Similarly, total neutrophils and eosinophils were increased only within the two highest dose detergent treatment groups. This could be a result of immune and allergic response to the toxic components of the detergent. The increased neutrophil production indicates a stimulation of the immune system which protects the mice against infection that might have been caused by chemical and also secondary infections. This may be directly proportional to the severity of the causative stress condition, may be attributed to an increase in neutrophil mobilization.

Although there were no previous findings for the effect of detergents on liver enzymes, there were limited reports on the tissue and organ damages. Ayandiran et al. (2009) previously reported histopathological changes in muscle tissues of detergent exposed catfish. Ogundiran et al (2010) also reported a multi-organ damages due to detergent exposure in catfish. Similarly, heart, lung and liver damage was apparent in mice treated with detergent solutions (Yahaya et al. 2011). Biochemical markers such as ALP and AST are often used as basis for the assessment of toxicity of an organ. An increase in ALP levels is usually a sign of non-specific tissue irritation and usually implies a toxic effect on the liver with bile duct alterations. The liver, kidney, heart, brain, and skeletal muscle tissues are the major sources for AST. Accordingly, a raise in AST activity may possibly be the result of liver, heart or muscle damage. Therefore, higher blood concentrations of ALP and AST in treated mice suggest liver damage, as was paralleled with decreased plasma protein levels.

CONCLUSIONS

In conclusion, our results suggested that especially the higher doses used in the current study could be toxic and cause health risks to mice. It will be worth investigating further as to if the parameters studied herein returned to baseline, upon the discontinuation of detergent exposure.

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