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Effects of oil plastic extract on mice

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In the plastics industry, various chemical additives are used to improve certain properties of plastics. Some of these chemicals, that might be toxic, have been proved to leach from the plastic containers and mix with their contents such as food oils, beverages, drugs, etc. Locally manufactured polyvinyl chloride (PVC) jerrycans were bought from the market and cut into small chips of 0.5 cm in the larger dimension. Four grams of chips were extracted with 20 ml cottonseed oil in the autoclave for 1 h at 121°C. The extract was prepared daily and given orally to adult MFI mice in a dose of 10 ml kg $^{-1}$ body weight. Pure cottonseed oil was prepared under the same conditions and given in a dose of 10 ml kg $^{-1}$ body weight to the control group. Treatment of both groups continued for 1 month. Each group comprised 60 animals, regardless of sex. Effects of the oil plastic extract were observed on blood elements, serum transaminases (aspartate aminotransferase, AST, and alanine aminotransferase, ALT), organ-to-body weight of the liver, kidneys and brain, and the nervous system (effects on the neuromuscular junction and analgesia, using the Rota-Rod® treadmill 'RRT', and the hot plate, respectively). All the results were subjected to Student's *t*-test. The results showed that the extract induced significant effects: an increase in the activities of AST ($p < 0.001$) and ALT ($p < 0.02$), an increase in the mean corpuscular haemoglobin concentration (MCHC) ($p < 0.01$), and the monocyte count ($p < 0.01$). It decreased the white blood cell count (WBC) ($p < 0.01$), the mean corpuscular volume (MCV) ($p < 0.05$), and the lymphocyte count ($p < 0.05$). It also reduced the weight of the liver ($P < 0.01$), kidneys ($P < 0.05$), and the endurance time on the RRT ($p < 0.001$).

Keywords: oil plastic extract; toxic effects; mice; PVC.

Introduction

Plastics are man-made polymeric materials made by the polymerization of various organic monomers. It is well known in the plastics industry that various chemical agents (additives) have to be blended with the main polymer to produce a plastic of certain physical and mechanical properties. These additives include plasticizers, ultraviolet-ray absorbers, stabilizers, filling agents, colorants, and flame retardants (Brydson 1982).

These materials are being introduced into countless human applications, such as the packaging of foodstuffs, food oils, and beverages. They are also used in the fabrication of numerous items and devices used in the medical and paramedical fields (Halpern and Tong 1989).

It has been proved, through the work of many researchers, that some of these additives or their reaction products, in addition to the unpolymerized molecules, are leachable from the parent

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plastic. Preda *et al.* (1983) investigated the migration of colorants from coloured polyethylene, polystyrene and polyvinyl chloride (PVC) plastics which are used for the manufacturing of containers, wraps and bags, utilized for the packaging or storage of different foodstuffs. They indicated that the extracts prepared from such plastics contained amounts of cadmium exceeding the sanitary levels. The term 'indirect food additives' was coined to indicate the migration of these chemical agents from household plastic containers and bags to their food contents. In the USA such agents were estimated to number 3000 (Information Service 1985).

Gramshaw *et al.* (1995) have analysed the extracts of four types of plastics, usually used in food packaging, and identified a number of chemicals with the potential to migrate into food. PVC bottles, used for retail beverages, were analysed by Castle *et al.* (1996) for PVC unpolymerized molecules. They could detect vinyl chloride tetramer in the plastic extracts they analysed, and indicated that this molecule has the potential for migration from plastic packaging. Butyltin compounds (BTs), such as mono-, di-, and tributyltin, which are widely used as stabilizing agents in plastics, were detected by Takahashi *et al.* (1999) in the livers of humans and other mammals. When these researchers analysed some plastic products that are used in food processing, such as baking parchment and gloves made from siliconized paper and polyurethane, respectively, they found elevated concentrations of BTs in these plastics. They concluded that the contamination in the investigated liver samples originated from the migrating BTs into the foodstuffs in contact with these plastics. Di-BT was reported by Ema *et al.* (1996) to be teratogenic to rat embryos and affects the organogenesis in all stages of embryo growth.

The problem of additive leaching from plastics has also been faced in the medical field, where various plastic items are used. Examples include implants, blood bags, tubing and many other devices which have direct contact with patients (Sevela *et al.* 1996).

Mettang *et al.* (1996) analysed the serum of patients, undergoing peritoneal dialysis (PD) for the plasticizer diethylhexyl phthalate (DEHP) and its hydrolysis products. They obtained high concentration levels for DEHP and phthalic acid. They indicated that the source of these chemicals is the plastic material of the bags used to package the PD solution. Blood contamination with these leachables in haemodialysis patients is worse than that which occurs to PD patients, particularly when PVC tubing is used in the haemodialysis unit, due to the migrating plasticizers (Nassberger *et al.* 1987). The release of chemical additives from the plastic material of blood bags was studied by Racz *et al.* (1993). They reported that all the types of blood bags (from different manufactures) they investigated were leachable to DEHP, even at low temperature (4°C).

The migration of chemical agents from the parent polymer depends on the specific plastic, the nature of material in contact with the plastic, and the conditions of contact. In general, plastics show little tendency towards aqueous extraction, but oil media have greater affinities for organic molecules present in plastics (Al-Khatim 1999). However, extraction of plastics, to study the adverse health effects of their leachable chemicals, provides a better method to evaluate these man-made materials than other techniques (Dang *et al.* 1996).

Materials and methods

Preparation of the plastic extract

The plastic extract was prepared as described in the *United States Pharmacopoeia* (1989). A household-type PVC plastic jerrycan was obtained from the local market and cut into small chips of 0.5 cm in the larger dimension. Extraction was carried out in 50 ml Pyrex culture test

tubes (150 × 25 mm) with white composition liners obtained from Fisher Scientific, St. Louis, MO, USA (catalogue No. 14-932E). Four grams of the plastic chips was placed into a test tube and 20 ml of cottonseed oil was added. Another 50 ml test tube of the same type contained 20 ml of cottonseed oil only. Both test tubes were closed and placed inside the autoclave at 121°C for 1 h. The plastic extract and cottonseed oil were then allowed to cool down to room temperature. Extracts, together with cottonseed oil, were prepared daily and used within 1 h after preparation.

Preparation of animals

Adult MFI mice, weighing approximately 30 g, were used. Animals were kept in macrolone cages (27 × 21 × 14) cm), with sawdust bedding and given food and water *ad libitum*. The colony room was on a 12 h dark–light cycle and the temperature was controlled at 26 ± 2°C throughout the experiment. The animals were divided into two groups, each of 60 mice, and designated as 'treated' and 'control'. The plastic extract and the cottonseed oil were given orally, in a daily dose of 10 ml kg^{-1} body weight, to the treated and control groups, respectively. Treatment of both groups continued for 1 month, after which all animals were subjected to various toxicologic tests (see parameters below).

Parameters used

For every performed toxicologic test, 10 animals were used, regardless of sex, for observations of possible toxicity induced by the oil plastic extract. The following parameters were measured: blood elements (complete blood and differential counts), using a Coulter Counter (Model S Plus STKR, Coulter Electronics, Florida, USA), serum transaminases: aspartate aminotransferase (AST) and alanine aminotransferase (ALT), using Reflotron 1 (Model FTZ-No.B-203/85, Boehringer-Mannheim, Germany), the endurance times to assess the neuromuscular junction and analgesia, using the Rota-Rod® treadmill (RRT) (Model No. 58688, Ugo Basile, Comerio, Va., Italy) and the hot plate (Model-DS37, Ugo Basile, Comerio, Va., Italy), respectively, and the organ-to-body weights for the liver, brain and kidneys.

Results

The significant data are reported in Tables 1–5. The effects, induced by the orally administered oil plastic extract, were as follows: a decrease in the percentage weight of liver ($p < 0.01$) and

Table 1. Effects of cottonseed oil plastic extract or cottonseed oil (control), given orally to MFI adult mice (10 ml kg^{-1} per day) for 1 month, on the weight percentage of internal organs related to total body weight (mean ± SD)

Treatment	*Liver weight (%)*	*Kidney weight (%)*	*Brain weight (%)*
Control (10)	5.17 ± 0.52	1.76 ± 0.28	1.49 ± 0.27
Treated (10)	4.64** ± 0.22	1.51* ± 0.22	1.51 ± 0.41

Notes:
() Number of animals per group.
* Significant difference from the control ($p < 0.05$).
** Highly significant difference from the control ($p < 0.01$).

Table 2. Effects of cottonseed oil plastic extract or cottonseed oil (control), given orally to MF1 adult mice (10 ml kg^{-1} per day) for 1 month, on the blood enzymes of aspartate aminotransferase (AST) and alanine aminotranferase (ALT) (mean ± SD)

Treatment	*AST (U/L)*	*ALT (U/L)*
Control (10)	109.31 ± 34.89	29.72 ± 9.09
Treated (10)	352.28*** ± 42.91	47.08** ± 17.28

Notes:
() Number of animals per group.
** Highly significant difference from the control ($p < 0.02$).
*** Very highly significant difference from the control ($p < 0.001$).

kidneys ($p < 0.05$) (Table 1); an increase in the activity of serum AST ($p < 0.001$) and ALT ($p < 0.02$) (Table 2); a decrease in the white blood cell (WBC) count ($p < 0.001$) and the mean corpuscular volume (MCV) ($p < 0.05$), accompanied by an increase in the mean corpuscular haemoglobin concentration (MCHC) ($p < 0.01$) (Table 3). A differential count of blood showed a decrease in the lymphocyte count ($p < 0.05$) and an increase in the monocyte count ($p < 0.001$) (Table 4). The endurance times on the RRT also showed a significant decrease ($p < 0.001$, Table 5). All data were subjected to statistical analysis using Student's *t*-test.

Discussion

The migration of some ingredients from the plastic material, by way of oil extraction, might be behind the aforementioned significant outcomes. It was also possible to differentiate the clear cottonseed oil in the control tube from the slightly turbid extract in the other tube, which suggests that plastic leaching occurred.

Since the liver is the main organ involved in the metabolism of xenobiotics and the kidney is the main site for the excretion of their metabolites (Murray 1996, Klaassen 1986), these two vital organs are prone to hepatotoxicity and nephrotoxicity, respectively, which are induced by many chemical agents. Malfunction of these organs, due to these toxic effects, might be associated with mass reduction of the organ as a result of tissue destruction (Arcadi *et al.* 1998). Damage of liver cells is followed by the release of aminotransferases, AST and ALT, into the circulation (Du *et al.* 1995). Al-Hachim and Al-Khatim (1997) reported similar results when they studied the aqueous plastic extract of the same plastic material. Al-Khatim (1999) has reported the elevation of aminotransferase activity and serum non-protein nitrogenous compounds in the progeny of mice that were treated with peritoneal dialysis solution (PDS) stored in plastic bags. He ascribed these maternally induced hepatotoxic and nephrotoxic effects in the offspring to the leachable chemicals that migrated from the plastic material of the bag to the PDS stored therein. The results, indicated in Tables 1 and 2, confirm the findings of these researchers. Moreover, PVC plastic, the subject of this study, might be more harmful owing to the presence of plasticizers and vinyl chloride monomers and oligomer that are mostly included in the leachable agents (Castle *et al.* 1996; Sevela and Gajduskova 1996).

The results also show significant reduction in the WBC count (Table 3), with significant increase in the monocyte count (Table 4). Such effects might have been induced by the action of these leachables on the bone marrow. Many chemical agents lead to the failure of this organ

Table 3. Effects of cottonseed oil plastic extract or cottonseed oil (control), given orally to MFI adult mice (10 ml kg^{-1} per day) for 1 month, on complete blood count (CBC) (mean ± SD)

Treatment	*WBC* ($\times 10^{12} l^{-1}$)	*RBC* ($\times 10^{12} l^{-1}$)	*Hgb (g %)*	*HCT ratio*	*MCV (fl)*	*MCH (Pg)*	*MCHC (g %)*	*RDW*
Control (10)	4.75 ± 0.75	7.62 ± 0.56	13.32 ± 1.23	0.39 ± 0.03	51.09 ± 0.63	17.46 ± 0.72	34.20 ± 1.14	34.58 ± 2.58
Treated (10)	2.66*** ± 0.32	7.47 ± 0.63	12.69 ± 1.79	0.35 ± 0.06	48.76* ± 2.52	17.78 ± 0.94	36.48** ± 1.75	35.05 ± 2.68

Notes:
() Number of animals per group.
* Significant difference from the control ($p < 0.05$).
** Highly significant difference from the control ($p < 0.01$).
*** Very highly significant difference from the control ($p < 0.001$).

Table 4. Effects of cottonseed oil plastic extract or cottonseed oil (control), given orally to MFI adult mice (10 ml kg^{-1} per day) for 1 month, on leucocyte differential count (mean ± SD)

Treatment	*Lymphocytes (%)*	*Monocytes (%)*	*Granulocytes (%)*
Control (10)	80.30 ± 3.23	2.60 ± 1.10	17.11 ± 3.48
Treated (10)	76.78* ± 5.75	8.84*** ± 2.91	14.39 ± 5.03

() Number of animals per group.
* Significant difference from the control ($p < 0.05$).
*** Very highly significant difference from the control ($p < 0.001$).

to deliver normal formed elements or normal numbers of formed elements (Smith 1986). It has been shown that the monocyte count increases after the ingestion of some chemicals (Gallo and Stevens 1982). Monocytosis, shown in Table 4, apparently indicates the role of active phagocytosis as a means of body defence against such foreign compounds (Guyton, 1987). The significant decrease ($p < 0.05$) in the MCV, shown in Table 3, indicates microcytosis, resulting from variation in the RBC size (anisocytosis) that is normally associated with disturbed erythropoiesis in the bone marrow (Jones and Wickramasinghe 1991). The increased MCHC ($p < 0.01$), with normal MCH, is characteristic of spherocytosis (poikilocytosis) which might be induced by chemical injury in the bone marrow (Davidsohn and Nelson 1974, de Gruchy 1978).

The leachables in the plastic extract might also have affected the neuromuscular junction, probably due to the damage of synaptic clefts and terminals of myelinated axons of the motor nerves which are uniquely vulnerable to several toxic chemicals (Nakajima 1993; Norton 1986). The skeletal muscle weakness displayed by the significant reduction in the endurance time, recorded for the treated animals on the RRT (Table 5), is mostly indicative of this effect.

Conclusions

This study reveals that the problem of plastic leaching still requires more attention by researchers. The various health effects, associated with these leachables, as shown by this work, may indicate that this phenomenon of additive migration is not exclusive to a single chemical agent, but migration might include a group of additives. Some leachables and/or their metabolites have had their impact on the liver cells, as indicated by the increase in transaminase

Table 5. Effects of cottonseed oil plastic extract or cottonseed oil (control), given orally to MFI adult mice (10 ml kg^{-1} per day) for 1 month, on the endurance time (seconds), recorded on the Rota-Rod® treadmill (RRT) (mean ± SD)

Control (10)	*Treated (10)*
267.39 ± 51.93	161.78*** ± 16.91

Notes:
() Number of animals per group.
*** Very highly significant difference from the control ($p < 0.001$).

activity. It is probable that the effect on the kidneys might have arisen from such metabolites deriving from these leachables. On the other hand, the effects on haemopoiesis and the myoneural junction, as indicated by this study, suggest the diversity of the leaching agents, since several xenobiotics are expected to be extracted from the plastic material with oil media. Thus, the extraction method applied in this study seems, in our opinion, suitable and practical to evaluate the compatibility of plastics that are used for food packaging and medical applications. Scientists are also invited to study the metabolic fate of various chemical additives proved to leach from plastics.

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