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Prenatal Effects of Aqueous Plastic Extract on Offspring

Key Words
Aqueous plastic extract
Prenatal effects
Mice
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Abstract
There is a huge plastic industry in the Kingdom of Saudi Arabia, where plastic wares are widely used. Locally manufactured jerricans were brought from the market and cut into small chips of 0.5 cm in the larger dimension. Four gram chips were extracted with 20 ml normal saline solution in the autoclave for 1 h at 121 °C. The extract was prepared daily and administered at a dose of 20 ml/kg/day i.p. to MFI mice during gestation. The control group was given normal saline. Both groups included at least 20 pregnant mice each. The prenatal effects of the extract were investigated with respect to the gestational period, neonatal mortality, body weight, body growth rate, body length, eye opening, weight of the internal organs, blood enzymes, and nervous system (neuromuscular junction analgesia and behavior) by using the accelerating Rota-rod treadmill (Ugo Basile, Varese, Italy), a hot plate and an automatic reflex conditioner of the offspring. All the results were subjected to t test. The results indicated that prenatally administered aqueous plastic extract increased the percentage of liver weight ($p < 0.05$), raised the aspartate aminotransferase activity ($p < 0.01$) and alanine aminotransferase activity ($p < 0.05$), reduced the gestational period ($p < 0.01$), reduced the body weight at birth ($p < 0.01$), reduced the body growth rate ($p < 0.01$, $p < 0.05$), and reduced the endurance time on the Rota-rod treadmill ($p < 0.01$, $p < 0.05$).

Introduction

The use of polymeric materials as components of household appliances as well as medical and dental prostheses and devices has increased exponentially over the past two decades. More than 175 industrial firms have been established in the Kingdom of Saudi Arabia for the production of synthetic polymers. The total production of plastic by these firms is estimated to be approximately 500,000 t/year [1].

In the past, much attention given to plastics was directed at their chemical, physical and mechanical properties, since knowledge in these specific areas is required to develop suitable products for a specific application. More recently, however, attention has been focused on the potential toxic liability of these polymeric materials, particularly when they may be used for medical and dental prostheses and household appliances.

Plastic household appliances are widely used and available in almost every household in the Kingdom of

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Saudi Arabia. Since these polymeric materials are prepared from monomers and other chemical additives which may be toxic, the toxic liability of these agents in Saudi-made plastics is receiving considerable attention by local health authorities. Hence it is becoming vital to investigate and evaluate the health hazards of the locally produced plastic to improve its quality and to protect the health of the Saudi population.

Methods

Preparation of the Plastic Extract

The plastic extract was prepared as described in the United States Pharmacopeia [2]. A plastic jerrican of the household type was 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 line obtained from Fisher Scientific Company, St. Louis, Mo., USA (catalog No. 14-932E). Two grams of the plastic chips was placed in a tube and 10 ml of normal saline was added. Another 50-ml test tube of the same type contained normal saline only. Both test tubes were closed and placed inside the autoclave at 121°C for 1 h. The plastic extract and normal saline were then allowed to cool down to room temperatures. Extracts, together with normal saline, were prepared daily and used within an hour after preparation.

Preparation of Animals

Three-month-old MFI, weighing approximately 25 and 30 g, were used. The females were mixed with males at 7 PM, and the vaginal plug was observed on the next morning at 8 AM to determine the first day of pregnancy. Each pregnant animal was then kept separately in a macrolone cage (27 × 21 × 14 cm), with sawdust bedding and was allowed food and water ad libitum. The colony room was on a 12-hour dark-light cycle and the temperature was controlled at 26 ± 2°C throughout the experiment. Two groups of 20 pregnant mice each were designated as 'treated' and 'control' and were given the plastic extract and saline solution, respectively, intraperitoneally. During the first 3 days after delivery, the litter size was subjected to culling so that every mother had 8 babies. After 1 month the offspring were weaned; each one was placed in a separate cage, with sawdust bedding and food and water ad libitum. These offspring were subjected to various toxicological tests when they were 1 month old.

Parameters

For every performed toxicological test, 10 offspring were selected randomly for observations of the toxicity possibly induced by the aqueous plastic extract. The following parameters were monitored: gestational period, litter size, and body weight of the offspring at delivery. Time of eye opening, weight of internal organs (liver, kidneys and brain), blood composition, and skeletal malformation of the offspring were also reported. Weight of the liver, kidneys, and brain were recorded as the percentage of total body weight for each offspring in the group selected for this test. Blood enzyme activities for aspartate transaminase (AST) and alanine transaminase (ALT; in units/liter) besides the bilirubin concentration (mg%) were obtained by analyzing the fresh blood samples withdrawn from the offspring by heart puncture. The samples were analyzed by the Reflotron® I (model FTZ-No. B-203/85, Boehringer-Mannheim, Germany). Muscular malformation was observed by recording the endurance time (in seconds) for each animal. This test was carried out via the accelerating Rota-rod treadmill for mice. The offspring was put on the rotating rod. It tried not to fall down the rod by walking against the direction of the rod motion until it reached the threshold of fatigue and fell down, and automatically the counter was disconnected. The time that was utilized by each offspring to resist falling down from the accelerating Rota-rod treadmill was recorded to compare between the treated and control groups.

Table 1. Prenatal effects of aqueous plastic extract or normal saline (control) given intraperitoneally to MFI mice (20 ml/kg/day) on the percentage of weight of internal organs to total body weight in the 1-month-old offspring

Treatment	Liver wt. %	Kidney wt. %	Brain wt. %
Control (n = 10)	4.96 ± 0.31	1.37 ± 0.15	1.66 ± 0.24
Treated (n = 11)	5.39 ± 0.55*	1.29 ± 0.16	1.59 ± 0.28

Data are presented as mean ± SD.
* $p < 0.05$, significantly different from the controls.

Table 2. Prenatal effects of aqueous plastic extract or normal saline (control) given intraperitoneally to MFI mice (20 ml/kg/day) on the blood levels of AST, ALT and bilirubin in the 1-month-old offspring

Treatment	AST, U/l	ALT, U/l	Bilirubin mg%
Control (n = 10)	94.7 ± 29.2	26.1 ± 6.2	0.92 ± 0.52
Treated (n = 11)	171.8 ± 12.5**	40.4 ± 11.7*	0.88 ± 0.45

Data are presented as mean ± SD.
* $p < 0.05$, significantly different from the controls.
** $p < 0.01$, highly significant difference from the controls.

Results

All the results were subjected to t test. The significant data are reported in tables 1–5, and indicate that the effects of the prenatally administered aqueous plastic extract on the offspring increased the percentage of liver weight ($p < 0.05$, table 1), the AST activity ($p < 0.01$) and ALT activity ($p < 0.05$, table 2), and reduced the gestational period ($p < 0.01$, table 3), the body weight at birth ($p < 0.01$, table 4), the body growth rate ($p < 0.01$, $p < 0.05$, table 4), and the endurance time on the Rota-rod treadmill ($p < 0.01$, $p < 0.05$, table 5).

Table 3. Prenatal effects of aqueous plastic extract or normal saline (control) given intraperitoneally to MFI mice (20 ml/kg/day) on the gestational period (in days)

Control (n = 10)	Treated (n = 10)
19.2 ± 0.5	18.0 ± 0.0**

Data are presented as mean ± SD.
** $p < 0.01$, highly significant difference from the controls.

Table 4. Prenatal effects of aqueous plastic extract or normal saline (control) given intraperitoneally to MFI mice (20 ml/kg/day) on the growth rate of offspring (in grams)

Treatment	1 day old	5 days old	10 days old	15 days old	20 days old
Control (n = 16)	1.94 ± 0.24	4.89 ± 0.33	7.47 ± 0.36	10.04 ± 0.98	11.60 ± 0.61
Treated (n = 15)	1.03 ± 0.24**	3.77 ± 0.14**	6.77 ± 0.25*	8.87 ± 0.35*	10.49 ± 0.48*

Data are presented as mean ± SD.
* $p < 0.05$, significantly different from the controls.
** $p < 0.01$, highly significant difference from the controls.

Table 5. Prenatal effects of aqueous plastic extract or normal saline (control) given intraperitoneally to MFI mice (20 ml/kg/day) on the Rota-rod treadmill endurance time (in seconds) in the 1-month old offspring

Treatment	31 days old	32 days old	33 days old	34 days old	35 days old	36 days old
Control (n = 10)	118.0 ± 35.7	209.1 ± 70.7	218.1 ± 76.4	170.7 ± 108.9	226.8 ± 111.8	254.3 ± 79.7
Treated (n = 10)	31.2 ± 16.24**	22.9 ± 12.7**	58.3 ± 24.6**	88.0 ± 20.8*	117.2 ± 26.9*	106.9 ± 25.6**

Data are presented as mean ± SD.
* $p < 0.05$, significantly different from the controls.
** $p < 0.01$, highly significant difference from the controls.

Discussion

In the plastic industry, various chemical additives are added to import sepcific properties of plastics [3]. Some of these chemicals, additives and/or monomers, that might be toxic [4, 5] have been proved to leach from the plastic wares or containers and mix with their contents such as food, drugs, water or beverages [6]. These leached chemicals might be a health hazard for human beings and other organisms [7–9].

The clear prenatal effects of the aqueous plastic extract on mice as indicated in tables 1–5 are an indication that some chemicals passed through the placenta, subsequently influencing the physiological functions of the embryo/offspring. These results confirm the findings of various researchers [7, 9, 10].

The significant increase in percentage of liver weight (table 1) that is caused by prenatally administered aqueous plastic extract might be due to hepatic cell proliferation, which might be caused by the extracted plastic additives and/or monomers such as vinyl chloride [9–11]. This result is also confirmed by the rise of AST and ALT blood levels (table 2). These leached compounds in the plastic extract solutions have a cytotoxic effect [4] on the liver and thereafter caused liver injury. The hepatic intracellular enzymes AST and ALT had been released into the circulation, which significantly altered their activity levels in the blood stream as indicated in table 2. In addition to these vivid findings, these leached plastic products might also cause ultrastructural changes in hepatocytes [10], which will be subject to further investigation in our laboratory.

Aldyreva et al. [12] indicated that the plastic additives phthalates can be responsible for causing a tendency to miscarriage in women. In our study, the aqueous plastic extract caused a significant reduction in the gestational period in mice (table 3), confirming the finding of Aldyreva et al. [12]. This reduction might be caused by stimulating the release of oxytocin from the posterior pituitary gland via the extract components, additives and/or monomers.

Various prenatally administered xenobiotics reduce the body weight at birth [13] and also depress postnatal growth and development of the offspring [14]. The prenatally administered xenobiotics from plastics also caused significant reduction of body weight at birth and significantly depressed the postnatal development of the offspring (table 4). There is no doubt that the extracted plas-

tic additives and the monomers can transfer through the placenta [7, 15, 16] to the fetus and are taken in by the offspring via mother's milk [17]. This indicates that plastic products, additives and/or monomers administered prenatally have effects on the fetus and the offspring via both placental transfer and milk secretion.

Singh et al. [18] showed the embryofetal toxicity and teratogenic effects of a group of methacryclic esters in rats. At one or more of the dose levels, each ester produced gross skeletal malformation. These effects were dose-related. In the present study, when the 1-month-old mice treated prenatally with aqueous plastic extract were subjected to an accelerating Rota-rod treadmill for 6 consecutive days, the results, as indicated in table 5, showed that the endurance time of the treated group was significantly less than that of the controls. This reduction in endurance time might be caused by skeletal malformation. Thus the aqueous plastic extract components possibly included some teratogenic chemical agents. This teratogenic effect for the aqueous plastic extract was confirmation for the results of other workers [7, 15, 16, 18].

The evidence presented here strongly suggests that human health may be jeopardized by plastic products. Finding a solution to this problem is, however, extremely difficult and is laden with both moral and economic considerations. The plastic industry is a multimillion-dollar industry, and it is too much to expect plastic products to be removed from human usage in the near future. Realizing this, many scientists have called for the development of plastic products without the leaching problem described [19, 20].

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