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EFFECT OF CARROT AND WHEAT GERM OIL SUPPLEMENTATION ON RATS EXPOSED TO BENZENE

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ABSTRACT

Benzene is an aromatic hydrocarbon. It gives rise to the production of oxygen radicals or reactive oxygen species (ROS), which are the means of the metabolic activation of benzene and are the source of its toxicity. This study was conducted to assess the ability of some food stuffs such as carrot and wheat germ oil to protect against benzene toxicity.Experiments were carried out on albino rats injected with benzene (0.5ml/kg body weight ip) and given diet supplemented with carrot and wheat germ oil. The dietary consumption and growth rate were measured. Several biochemical parameters representing antioxidant status were followed. The results showed that food intake and body weight gain of rats injected with benzene were significantly lower than that of control rats. Plasma malondialdehyde was increased and the levels of vitamins A & E and the activity of the antioxidant enzymes were decreased in rats injected with benzene. Supplementation with carrot and wheat germ oil caused a significant decrease in plasma malondialdehyde and significant increase in the level of vitamins and the antioxidant enzymes. The histopathological examination of the liver tissues of animals injected with benzene showed different lesions but supplementation with carrot and wheat germ oil caused an improvement in liver as compared with the benzene group. This study indicates that the toxic effect of benzene exposure can be partially corrected by food ingredients such as carrot and wheat germ oil. It is recommended to be given to individuals who are exposed to environments polluted with benzene.

Keywords: benzene, exposure, carrot, wheat germ oil, rat.

INTRODUCTION

Many environmental pollutants can cause oxidative damage to the biological systems. Benzene is an environmental pollutant absorbed and oxidized in the liver after its inhalation, oral or dermal exposure. Long term animal studies showed that benzene causes tumors at multiple sites in mice and rats (Huff, *et al.*, 1989).

The major determinants of benzene toxicity have been suggested that this solvent gives rise to the production of oxygen radical or ROS (Parke, 1996).

Natural antioxidants such as vitamin E, A, β-carotene and vitamin C play a central role in promoting defense mechanism against oxidative stress (Halliwell *et al.*, 1992 and Frei, 1994). To minimize the damaging effect of ROS, it is important to promote the function of the enzymatic and nonenzymatic regulating system present in the body. Glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) are among the enzymes that play a critical role for depriving the cell from these ROS (Urso and Clarkson, 2003 and Valko *et al.*, 2006).

Numerous studies support the view that diets rich in fruits and vegetables may protect against various diseases, especially cardiovascular diseases (CVD) and cancers (Potter & Steinmetz, 1996; Riboli, *et al.*, 1996 and McDermott, 2000).

This study was carried out to evaluate the potential role of some food staffs that are rich in antioxidant, such as carrot and wheat germ oil on the antioxidant vitamins, antioxidant enzymes activity, lipid peroxide levels and protection of cells against oxidative damage in rats due to benzene toxicity.

Materials and Methods

The ingredients used in the present investigation are: Dry Skimmed milk (vitamins-free) was obtained from Misr Dairy Company, Egypt, wheat germ oil (WGO) was obtained from Mobaco Company, Egypt and Carrot, purchased from the local market.

Treatment of Carrot Samples:

Carrot samples were washed in cold water, crashed, and lypholized then ground to obtain suitable fine powder. Three samples were used for the determination of carotenoids using HPLC method according to Epler *et al.* (1993).

Animals:

Forty male Sprague Dawley rats (average weight 194 g), bred at the Central Animal House of the National Research Center, Dokki, Giza, Egypt. After an initial 24 h acclimatization period, all rats were given a standard diet for 1 wk. The animals were kept through the experimental period (9 weeks) under good ventilation and hygienic conditions, experimental diets and water were fed *ad-libitum*. Body weights were measured weekly, food intake was measured twice weekly and examined each day for general condition.

Diets:

The experimental diets contain 10% fat and 14% protein (Table 1). The diet contains adequate vitamins and minerals according to the American Institute of Nutrition (Reeves *et al.*, 1993). Diets supplemented with either carrot or wheat germ oil was given to rats three days before benzene injection.

Table 1: Composition of the experimental diets fed to rats over the 9-week study period

Diet component (%)	Diet 1 & 2	Diet 3	Diet 4
Skimmed milk	35	35	35
sucrose	10	10	10
Wheat germ oil	---	---	10
Sun flour oil	10	10	---
Cellulose	5	4	5
AIN-93 mineral mixture	3.5	3.5	3.5
AIN-93 vitamin mixture	1	1	1
Choline bitartrate	0.25	0.25	0.25
L-Cystine	0.18	0.18	0.18
Lyophilized carrot	---	3	----
Starch	35.1	33.1	35.1

Experimental design:

The animals were randomly divided into four groups of 10 rats each, having a mean weight within 194±15 gm. Animals were housed individually in stainless steel cages and benzene was intraperitoneally injected three times a week 0.5 ml per kg body weight in corn oil (200 μL/animal) according to Ahmad *et al.* (1994).

Group 1: Control rats were fed on the basal diet.

Group-2: Rats were treated with benzene and fed on the basal diet.

Group 3: Rats were treated with benzene and fed on diet supplemented with 3% lyophilized carrot powder containing about 5 times the recommended requirement of vitamin A.

Group 4: Rats were treated with benzene and fed on diet supplemented with 10% wheat germ oil which contains α-tocopherol about 10 times the recommended requirement.

The experimental period lasted for 9 weeks during which rats were weighed weekly. At the end of the experimental period, animals were fasted over night and blood samples were collected in heparinized tubes under slight diethyl ether anesthesia by open heart puncture. The collected blood was divided into 2 parts:

The first one was used for the estimation of Hemoglobin concentration (Hb) by the cyanmethemoglobin method according to Eagle Hemoglobin procedure (Van Kampen and Zijlstra, 1961). Hematocrit percent (Hct %) was measured, reduced glutathione (GSH) concentration was determined by the method of Beutler *et al.*, (1963) and glutathione peroxidase (GSHPx) was determined using Kit provided by WAK-CHEMIE Medical GMBH, Germany, according to (Ammerman *et al.*, 1980).

The second part was centrifuged at (1500 xg) for 15 min to obtain total blood plasma. The plasma was then aliquoted and stored at − 20 °C until used for the analysis. Plasma malondialdehyde (MDA) was estimated according to Satoh (1978). Iron & total iron binding capacity (TIBC) and ferritin were determined using the commercial kit provided by Biodiagnostic, Cairo, Egypt. Vitamin A and β-carotene were determined according to Neeld and Pearson (1963). Vitamin E was estimated using the method of Desia and Machilin (1985). The erythrocytes were washed three times in cold normal saline (0.9% Na Cl). The heamolysate was used for the assay of catalase (CAT) according to Beers and Sizer, (1952) and superoxide dismutase (SOD) using kit provided by WAK-CHEMIE Medical GMBH, Germany (Arthur and Boyne, 1985).

Histopathological examination of the liver tissues

Liver specimens were removed and rapidly washed in saline solution to remove the blood. The specimens were rapidly fixed in 10% neutral buffered formalin for 24 hr., then processed up to paraffin blocks and sections 6um thick were prepared and stained with hematoxylin and eosin, (Drury and Wallington, 1980) for histopathological studies.

Statistical Analysis

Results are expressed as means ± standard errors of means (SEM). Comparison between the means was accomplished using a one-way

ANOVA, followed by Duncan Multiple Range Tests for all variables (Duncan 1955). Differences between groups were considered significant at $p < 0.05$.

Results and Discussion:

In the present study, it was found that HPLC analysis of lyophilized carrot revealed that 100g carrot contains α-carotene (13.0mg); β-carotene (31.3 mg) equivalent to 22.68 mg vitamin A (equal to 75600 IU). Since one kg diet requires 4000 IU vitamin A, we added 30g of lyophilized carrot which provide 5 times the requirement.

The results showed that food intake and gain in body weight of rats injected with benzene were significantly lower than that of control rats (Table 2). Many animal studies reported that exposure to organic solvent reduced food intake and body weight gain in mice (Dempster *et al.*, 1984) and in rats (Moròn *et al.*, 2004 Saillenfait *et al.*, 2006), they reported that this effect may be due to loss of appetite. Diet supplemented with either carrot or wheat germ oil improved the food consumption, body weight gain and food efficiency ratio in rats injected with benzene. This shows that these supplements are able to improve the condition of benzene toxicity.

Table 2: Food intake (g), body weight gain (g), and feed efficiency ratio (FER) of control rats, benzene treated and supplemented groups.

Parameters	Control	Benzene group	Benzene + Carrot	Benzene + WGO
Food intake (g)	1285 ± 14.28^{b}	1146 ± 14.15^{a}	1176 ± 9.48^{a}	1179 ± 15.30^{a}
body weight gain (g)	114.7 ± 3.77^{c}	68.0 ± 3.89^{a}	$75.2 \pm 3.43^{a, b}$	$75.6 \pm 3.99^{a, b}$
FER	0.089 ± 0.003^{b}	0.060 ± 0.004^{a}	0.064 ± 0.003^{a}	0.064 ± 0.004^{a}

Values within a row with different superscripts are significantly different (P<0:05).

Rats injected with benzene, showed a significant increase in the liver weights of the benzene group compared to control group ($p<0.05$) (Table 3). This should be considered as a liver specific change that cannot be ascribed to reduction of body weight only (Bar, 1999). Heijne *et al.* (2005) reported that the increased expression of drug metabolism enzymes in the liver might be the most important reason for the relative increase of the liver weight. It was observed a decrease of kidney and spleen weights. These findings are in line with the previous work (Yamamura *et al.*, 1999). Rats given supplemented diets showed a significant decrease in the liver weight and relatively an increase in the weight of kidney and spleen (Table 3).

Table 3: Weights of liver, spleen, and kidney of control rats, benzene treated and supplemented groups.

Parameters	Control	Benzene group	Benzene + Carrot	Benzene + WGO
liver (g)	7.88 ± 0.260^{a}	8.76 ± 0.264^{b}	7.720 ± 0.293^{a}	7.72 ± 0.328^{a}
spleen (g)	1.19 ± 0.048^{c}	0.84 ± 0.015^{a}	$0.93 \pm 0.031^{a, b}$	0.96 ± 0.031^{b}
Kidney (g)	0.90 ± 0.033^{a}	0.81 ± 0.025^{b}	$0.88 \pm 0.018^{a, b}$	0.89 ± 0.023^{b}

Values within a row with different superscripts are significantly different (P<0:05).

Lower values of Hb, Hct, iron and ferritin were reported in rats exposed to benzene (Table 4). There have been numerous studies of benzene-induced hematotoxicity (Ahmad et al., 1994, d'Azevedo, *et al.*, 1996, Escorcia *et al.*, 1997 and Qu *et al.*, 2002). However, rats received diet supplemented with each of carrot or wheat germ oil showed a significant increase in these parameters. The level of plasma TIBC was higher in benzene group than the control (p<0.001). Supplemented diets corrected this parameter compared to benzene group. Carrot is a valuable source of carotenoids (Alasalvar *et al.*, 2001), which provide rats with Vitamin A. Many studies showed a positive effect of vitamin A supplementation on Fe status in humans and animal models, (Garcı´a-Casal *et al.*, 1998 and Roodenburg *et al.*, 1996). Also carrot contains vitamin C, which has been shown to enhance Fe uptake in humans and in cell culture models (Sandberg, 2002 and Engle-Stone *et al.*, 2005). Wheat germ oil when given in combination with benzene at appropriate dose, it increases the antioxidant potential of the animals and decreased the toxic effect of benzene.

Table 4: Levels of Hemoglobin (g/dl), Hematocrit (Hct %), Iron (µg/dL), Ferritn (µg/L) and TIPC (µg/dL) of control rats, benzene treated and supplemented groups.

Parameters	Control	Benzene group	Benzene + Carrot	Benzene + WGO
Hemoglobin (g/dl)	14.02 ± 0.242^{b}	12.85 ± 0.297^{a}	14.01 ± 0.179^{b}	13.92 ± 0.172^{b}
Hematocrit (Hct %)	44.0 ± 1.12^{b}	39.6 ± 1.46^{a}	43.7 ± 0.87^{b}	43.5 ± 1.06^{b}
IRON (µg/dL)	108.9 ± 2.61^{b}	92.7 ± 3.53^{a}	103.1 ± 2.51^{b}	101.8 ± 2.00^{b}
FERRITIN (µg/L)	85.84 ± 6.54^{d}	52.24 ± 4.60^{a}	$69.09 \pm 3.44^{b,c}$	$60.62 \pm 4.76^{a,b,c}$
TIPC (µg/dL)	258 ± 2.83^{a}	347 ± 3.90^{c}	282 ± 5.82^{b}	292 ± 7.19^{b}

Values within a row with different superscripts are significantly different (P<0:05).

Rats injected with benzene showed an increase in MDA levels (p<0.01) accompanied with a decrease in the levels of scavenging enzymes SOD, catalase, glutathione peroxidase (GSH-Px) and GSH concentration than control group (table 5). The data are similar to those from other reports which indicated that benzene administration increased the level of MDA in albino rats (Pandya *et al.*, 1990 & Ahmad *et al.*, 1994). Also Chen, (1992) observed that in the workers exposed to benzene the content of serum MDA increased and the activities of erythrocyte SOD and erythrocyte GSH-Px were decreased. Our results showed that rats fed diet supplemented with carrot or WGO along with benzene had a reduction in the level of MDA (p<0.01). GSH concentration and the SOD, GSH-Px, CAT activity were improved (Table 5). These results are in agreement with that of Nicolle *et al.* (2003) who noticed a significant decrease in the urinary excretion of thiobarbituric acid reactive substances (TBARS) and reduced TBARS levels in the heart after feeding rats on carrot diet. Carrot contains arotenoids and other antioxidants such as vitamin E, vitamin C and phenolics such as *p*-coumaric, chlorogenic and caffeic acids (Alasalvar *et al.*, 2001). Antioxidants such as vitamin C, tocopherols, carotenoids and polyphenols are able to quench free radicals, together with the endogenous systems of defense. The strong antioxidant properties of β-carotene have been proven in several studies (Diplock, 1991 and Lomnitski *et al.*, 1993).

Wheat germ oil is unique among dietary supplements, it is highly rich in the most biologically active forms of naturally occurring vitamin E and mixed tochopherols (Sies and Stahl, 1995). Vitamin E act as inhibitor of oxidation processes in body tissues, it protects the unsaturated fat in the body from oxidation. It has been reported that oral administration of wheat germ oil efficiently saturates the body of rats with vitamin E and inhibits oxidation (Paranich *et al.*, 2000). These data were in agreement with many other reports (Ynal *et al.*, 1998; Bansal, *et al.*, 2005; Yousef *et al.*, 2006). thus vitamin E can be given as a nutritional supplement to reduce oxidative stress.

Table 5: Levels of malondialdehyde (MDA)(nmol/ml), reduced glutathione (GSH) (micromol/gHb), glutathione peroxidase (GSH-Px) (u/l Hb), catalase (ku/g Hb), and superoxid dismutase (SOD) (U/g Hb) of control rats, benzene treated and supplemented groups.

Parameters	Control	Benzene group	Benzene + Carrot	Benzene + WGO
MDA (nmol/ml)	4.62±0.217 [a]	6.82±0.14 [b]	5.22±0.231 [a]	5.08±0.207 [a]
Reduced glutathione (micromol/gHb)	31.08±0.874 [a]	24.91±0.968 [b]	31.10±0.815 [b]	33.99±.971 [c]
GSH-Px (U/L Hb)	4198±148.3 [a]	3762±169.5 [a]	4479±160.5 [b]	4643±144.9 [b]
Catalase (ku/g Hb)	58.04±1.15 [b]	50.38±1.81 [a]	59.29±2.11 [b, c]	64.23±3.30 [b, c]
SOD (U/g Hb)	735.6±30.09 [b]	629.6±27.77 [a]	745.5±27.95 [b]	751.7±36.17 [b]

Values within a row with different superscripts are significantly different (P<0:05).

Plasma vitamins A and E were significantly decreased in the benzene injected group compared with the controls (Table 4). However carrot supplementation caused a 23.26% elevation in vitamin A level and 14.8% in vitamin E compared to benzene group. This is parallel to the finding of Nicolle et al. (2003) who noticed that vitamin E level in the plasma of rats was increased after feeding carrot diet. β-carotene was not detected in the plasma of rats after given diet supplemented with carrot. The rat isn't able to absorb intact β-carotene, hence very little or even no intact β-carotene is taken up into the circulation (Ribaya-Mercado *el al.*, 1989). It has been reported that most of the absorbed vitamin A was obtained from that produced by cleavage of β-carotene in the intestinal mucosa (Krinsky *el al.*, 1990).

Supplementation with wheat germ oil (WGO) corrected the drop in plasma vitamin A that occurred due to treatment with benzene. It also provid the most biologically active forms of naturally occurring vitamin E and β-carotene (Krishnamurty *et al.*, 1982). The value reported for vitamin A (30.11 ug/dl) was near to the control (31.51 µg/dl). Also there was a significant improvement noticed in the level of vitamin E in the group given the supplemented diets compared to benzene group. Our result agrees with the finding of Ynal *et al.*, (1998) who found that the plasma α-tocopherol levels in benzene plus α-tocopherol group of Wister albino rats were significantly higher than in the control and benzene group.

Table 6: Levels of Vitamin A (μg/dl) and Vitamin E (mg/dl) of control rats and those treated with benzene and supplemented groups.

Parameters	Control	Benzene group	Benzene + Carrot	Benzene + WGO
Vitamin A (μg/dl)	31.51±1.07 ^{b}	25.97±0.95 ^{a}	31.79±1.28 ^{b}	30.11±0.86 ^{b}
VitaminE (mg/dl)	1.10±0.05 ^{c}	0.81±0.031 ^{a}	0.93±0.027 ^{b}	0.99±0.038 ^{b,c}

Values within a row with different superscripts are significantly different (P<0:05).

The microscopic examination of control liver of rats showed the common characteristics lobular organization. Each lobule is formed of cords of hepatocytes radiating towards a central vein. The hepatic lobules are separated by loose connective tissues at certain angles of the portal triad including branches of the portal vein, hepatic vein and bile duct (Fig. 1).

Examination of liver sections of rats receiving benzene showed periportal necrosis of the hepatocytes near the portal areas. The specimens also, showed dilated and congested portal vessels as well as mild areas of inflammatory cell infiltration especially in the vicinity of the portal veins and near the bile ductules. Some cells exhibited necrosis together with pyknosis of some nuclei. Slight haemorrhage was also noticed. Besides dilated sinusoids and the interlobular connective tissue showed marked thickening (Fig. 2). These results are in agreement with several results after exposure to benzene or its derivatives in mice (Szymanska, 1998), in rats (Madej *et al.*, 1987) and in workers exposed to benzene (Cotrim *et al.*, 2004). The resulting effect was due to the production of elevated amounts of oxidation products and conjugated dienes, which caused deleterious effects on the membranous components of hepatocytes.

Daily administration of carrot equivalent to 5 times the vitamin A requirement along with benzene showed that the liver appears more or less like normal except for single cells necrosis (Fig. 3). This similar to the finding by Nicolle *et al.* (2004) who reported that carrot ingestion lead to improvement of the antioxidant status in mice.

The histology of liver of rat given benzene and supplemented with WGO shows little necrosis and some inflammatory cells (Fig. 4), which indicates that the cellular recovery process was taking place but was not complete during the experimental period. It was shown that oral administration of wheat germ oil efficiently saturates the body with vitamin E and leads to inhibition of peroxidation in rats (Paranich *et al.*, 2000). The magnitude of the effects of WGO may not appear large but the experiments clearly indicate beneficial role of the WGO.

It is concluded from this work that benzene exposure results in varying degrees of oxidative stress with some tissue specific changes. So the present study highlights the protective role of some food stuffs such as carrot and wheat germ oil (WGO) in reducing the degree of oxidative stress induced by the environmental pollutants like organic solvents such as benzene.

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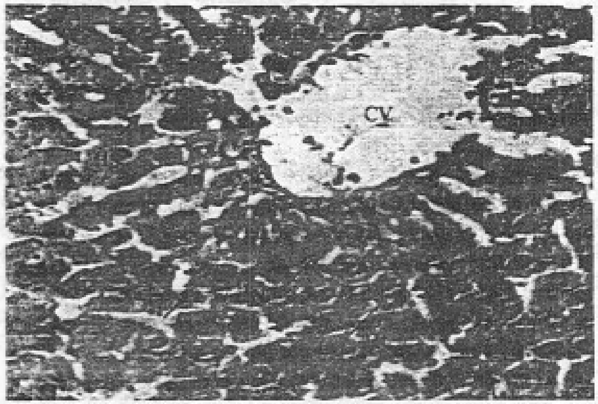

Figure (1): A photomicrograph of section of control liver showing the architecture of a hepatic lobule. The central vein (CV) surrounded by the hepatocytes (HC) with strongly eosinophilic granulated cytoplasm (CY) and distinct nuclei (N). Between the strands of hepatocytes the hepatic sinusoids (HS) are shown. (H & E stain-X 300).

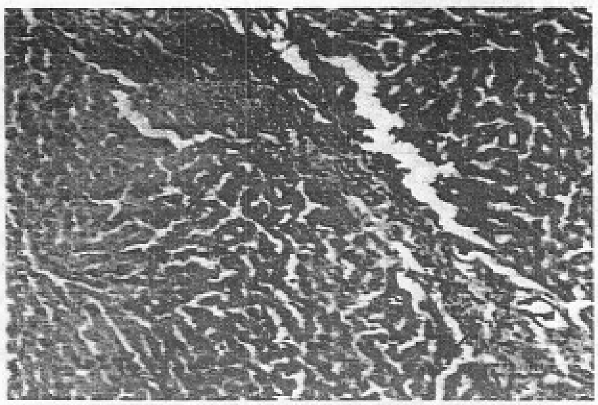

Figure (2): A photomicrograph of section of liver of rat injected with benzene showing focal necrosis (arrows), inflammatory infiltration, thickening of the interlobular connective tissue (long arrow) and the dilated and congested portal vein (arrow head). (H & E stain-X 300).

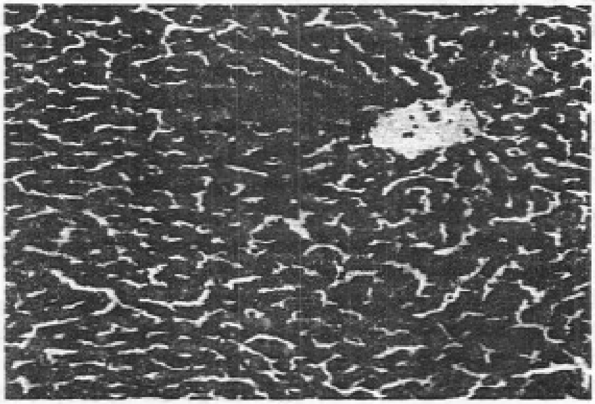

Figure (3): A photomicrograph of section of liver of rat injected with benzene and supplemented with lyophilized carrot shows that the structure appears more or less like normal except single cell necrosis (arrows).(H & E stain-X 300).

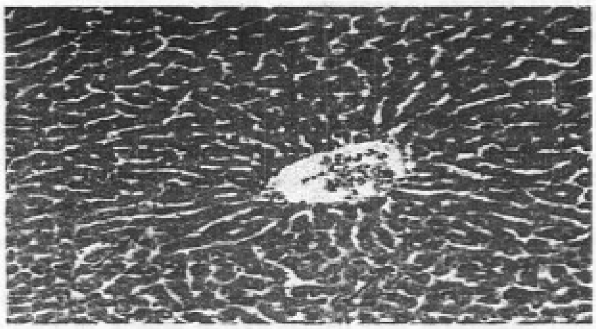

Figure (4): A photomicrograph of section of liver of rat injected with benzene and supplemented with wheat germ oil shows that the structure appears more or less like normal except single cell necrosis (arrows).(H & E stain-X 300).

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تأثير التعزيز بالجزر وزيت جنين القمح على الفئران المعرضة للبنزين

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يعتبر البنزين من أحد ملوثات البيئة و هو من أهم المذيبات المستخدمة في الصناعة. ويتعرض العمال أثناء إنتاج او إستخدام البنزين إلي مستويات مرتفعة منه و ذلك لسرعة تبخره.
يمتص البنزين و ينتشر سريعا في الجسم و يتحول الي مركبات متعددة في كثير من الأعضاء مثل الكبد و النخاع العظمي. و يتحول البنزين بفعل بعض الإنزيمات الموجودة بالجسم إلي مركبات وسطية نشطة قد تؤدي الي زيادة الشقوق الحره.

أجريت هذه الدراسة لمعرفة التاثير السمي للتعرض للبنزين علي الفئران. و تهدف إلي معرفة كفاءة الوجبات المعززة بالجزر وزيت جنين القمح في مقاومة سمية البنزين. تم حقن الفئران بالبنزين ثلاثة ايام في الاسبوع لمدة ٩ اسابيع (٠,٥ ملي /كجم من وزن الجسم داخل الغشاء البروتوني) وتقييم أثر التعرض للبنزين علي بعض التحاليل البيوكيميائية و التغيرات الهستوباثولوجية في كبد الفئران. وأظهرت النتائج إنخفاضا ذو دلالة إحصائية في كمية استهلاك الطعام مع زيادة في وزن الجسم و لوحظ أيضا انخفاض في نسبة الطعام المكافئة و أوزان (الطحال والكلي) وفيتامينات(أ , هـ) و الحديد والفرتين في البلازما والهيموجلوبين والهيماتوكريت والجلوتاثيون المختزل في الدم ونشاط لتزيمي الكتاليز وسوبر اكسيد ديسميوتز في المجموعة المعرضة للبنزين مقارنة بالمجموعة الضابطة. كما لوحظ وجود زيادة ذو دلالة إحصائية في اوزان الكبد و في كل من مالون دي ألديهايد (MDA) في البلازما و لم يكن هناك أي اختلاف في نشاط الجلوتاثيون بيرأكسيديز. حدثت تغييرات للخلايا الدالة علي الإلتهابات في مقاطع من كبد الفئران المعاملة بالبنزين.

أدت إضافة الجزر وزيت جنين القمح للوجبات التى تغذت عليها الفئران المحقونة بالبنزين إلى تحسن ملحوظ فى المدلولات التى تم دراستها .

تلقي هذه الدراسة الضوء علي الدور الوقائي لبعض المصادر الغذائية مثل الجزر أو زيت جنين القمح وذلك لتقليل الشقوق الحرة الناتجة عن ملوثات البيئة مثل المذيبات العضوية و منها البنزين.