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Modulation of di- (2- ethylhexyl) phthalate induced hepatic toxicity by *Apium graveolens* L. seeds extract in rats

G. C. Jain,* Hemant Pareek, B. S. Khajja, Kusum Jain, S. Jhalani, S. Agarwal and Sameer Sharma

Reproductive Physiology Section, Department of Zoology, University of Rajasthan, Jaipur - 302004, India.

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In the present study, the hepatoprotective activity of methanolic extract of Apium *graveolens* L. (celery) seeds was tested against Di-(2- ethylhexyl) phthalate (DEHP) induced hepatotoxicity in rats. Oral administration of DEHP (1000 mg/kg b.wt/day) for 6 weeks in rats caused a significant increase in the levels of serum marker enzymes like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatases (ALP) and the levels of total bilirubin and hepatic lipid peroxidation (TBARS). The levels of serum protein, hepatic glutathione (GSH) and ascorbic acid were decreased, administration of A. *graveolens* seeds extract (300 mg/kg b.wt./day p.o.) for 6 weeks results in a significant recovery of these biochemical parameters toward normalcy compared with the DEPC treated and control rats.

Key words: *Apium graveolens,* hepatic toxicity, di-(2- ethylhexyl) phthalate, hepatic marker enzymes.

INTRODUCTION

Di- (2-Ethylhexyl phthalate) (DEHP) is an aromatic di-ester widely used as plasticizer in polyvinyl chloride (PVC) resins for fabricating flexible vinyl products (Green et al., 2005). A substantial fraction of the world population is exposed to measurable levels of DEHP because of its wide spread use in consumer products. The primary routes of potential human exposure to DEHP are inhalation, ingestion, dermal contact and through medical devices (ASTDR, 1993) . Dietary exposure to DEHP occurs through migration of the plasticizer from packaging of food and from the environment where it exists as a contaminant in drinking water and aquatic food (WHO, 1992).

DEHP is a well-known hepatotoxin in animals. DEHP belongs to a class of chemicals called the peroxisome proliferators (PPs) (Moody and Reddy, 1978) as it stimulates hepatic peroxisomes proliferation and produce liver hypertrophy, hyperplasia and tumors (Rusyn et al., 2006).

Apium graveolens (Apiaceae) is one of the most well known plants used in the history of mankind as a medicament or a spice. It is commonly known as 'Ajmod' and the the fruits are popularly known as celery seeds. In the Indian traditional medicinal system, the seeds are used to treat bronchitis, asthma, liver and spleen diseases (Satyavati and Raina, 1976). Experimental studies have shown that A. *graveolens* possess antibacterial, nematicidal, antifungal, mosquitocidal (Momin and Nai, 2001a), anti- aggregation (Teng et al., 1985), anti-inflammatory and analgesic (Atta and Alkofahi, 1998; Lewis et al., 1985).

Hepatoprotective effect of methanolic extract of A. *graveolens* seeds has been reported in rats against various hepatotoxicants (Ahmed et al., 2002; Singh and Handa, 1995). Inhibitory effect of celery seeds extract on chemically induced hepatocarcinogenesis has been observed by Sultana et al. (2005). Besides, the extract of leaves has also shown its effectiveness in lowering oxidative stress induced by CCl_4 in mice. Moreover, it has also been reported to modulate reproductive toxicity induced by sodium valproate (Hamza and Amin, 2007).

Such widespread use of DEHP necessitates development of means to ameliorate liver toxicity induced by this chemical. Since plants are cheap and easily available sources of antioxidants and there are no reports available in the literature so far regarding herbal protection against DEHP induced hepatic toxicity, therefore the present in-

*Corresponding author. E-mail**:** jain_gc@yahoo.co.in. Tel.: +91-141-2369747

Table 1. Changes in relative liver weight and serum biochemical parameters in rats.

Treatments	Liver weight (mg/100 g b.wt.)	SGOT (unit/ml)	SGPT (unit/ml)	ALP (KA units)	Total bilirubin (mg/dl)	Total protein (mg/dl)
Control (vehicle)	3156 ± 84.23	62.93 ± 2.33	43.48 ± 3.66	12.60±0.93	0.83±0.93	7.12±0.27
DEHP (1000 mg/kgb.wt.)	3641 ± 67.02**	146.73 ± 7.34***	120.69 ± 5.83***	28.22±1.45***	1.69±0.08***	6. 31±0.15*
DEHP ± *A. graveolens* extract (300 mg/kg b.wt.)	3300 ± 111.36[a]	81.61±3.37[b]	68.82± 3.74[b]	15.78±1.03[b]	1.10±0.07[b]	6. 90±2.22

Values are mean ± SEM
Levels of significance: * p<0.05; ** p<0.01; ***p<0.001, DEHP treated rats compared with control rats
a p < 0.05,b p<0.001,DEHP + A. graveolens treated rats compared with DEHP treated rats.

vestigation was undertaken to examine the hepatomodulatory activity of *A. graveoelens* seed extract in rats against DEHP induced liver toxicity.

MATERIALS AND METHODS

Extract preparation

A. graveolens (apiaceae) seeds were procured from the authentic dealer and were botanically identified. These were dried in shade and subjected to soxhlet extraction for 30 h using methanol as solvent. The extract was filtered and concentrated to dryness under low temperature (40°C) and reduced pressure. The brown viscous mass so obtained was dissolved in distilled water and used for experimental study.

Di- (2-ethylhexylphthalate) (DEHP)

The chemical was procured from MERCK-Schuchardt, Hohenbrunn, Germany.

Animals

Colony bred adult male wistar rats, weighing 150 - 175 g, were used in the present study. They were housed in well-ventilated cages and maintained under standard environmental conditions (22 ± 3°C, 60 - 70% relative humidity, 12 h dark/light cycle) and provided standard rat feed (Lipton, India Pvt. ltd.) and water *ad libitum.* The Institutional ethics committee has approved the study.

Experimental Protocol

The rats were randomly divided into 3 groups of 7 animals each. Animals of group I served as normal control, received vehicle (corn oil) . Animals of group II received DEHP (1000mg/kg b.wt./day in 0.5 ml corn oil per rat) by intra-gastric intubation. Animals of group iii received *A. graveolens* extract (300 mg/kg b.wt./day p.o.) in addition to DEHP (1000 mg/kg b.wt./day). All the rats received treatment for 6 weeks duration.

Autopsy

After 6 weeks of treatment, the overnight fasted animals were sacrificed under light ether anesthesia. Their body weights were recorded. Blood was collected by cardiac puncture. Serum was separated and stored at -20°C for biochemical estimations. Liver was excised immediately, cleaned in ice-cold normal saline, blotted and weighed on digital balance. Pieces of liver tissue were fixed in buffered formaline for histopathological study. The remaining liver tissue was frozen at -70°C for biochemical analysis.

Biochemical parameters in serum

Serum samples were used for the estimation of the activities of serum glutamate oxaloacetate transaminase (SGOT) (Reitmen and Frenkel, 1957), and serum glutamate pyruvate transaminase (SGPT) (Reitmen and Frenkel, 1957), alkaline phosphatases (ALP) (Kind and King, 1954) and levels of total bilirubin (Malloy and Evelyn, 1937) and total protein (Lowry et al., 1951).

Biochemical parameters in liver

Fresh/frozen liver samples were analyzed for glycogen (Montgomery, 1957), lipid peroxidation (TBARS) (Ohkawa et al., 1979), glutathione (Moron et al., 1979) and ascorbic acid (Roe and Kuether, 1943).

RESULTS

There was a significant ($p < 0.01$) increase in the relative liver weight of DEHP treated rats as compared to normal rats, which was significantly ($p < 0.05$) restored by extract treatment. A significant increase in the level of SGOT ($p < 0.01$), SGPT ($p < 0.01$), and ALP ($p < 0.001$) was observed in serum of DEHP treated rats as compared to rats of normal group. Serum total bilirubin concentration also showed an elevation ($p < 0.001$) while the protein concentration declined significantly ($p < 0.05$). Co-administration of *A. graveolens* seeds extract significantly ($p < 0.001$) prevented the DEHP induced elevation in hepatic marker enzymes (SGOT, SGPT and ALP), total bilirubin as compared to DEHP treated control rats. (Table 1)

DEHP treatment caused a significant rise ($p < 0.001$) in the lipid peroxidation (TBARS) with a concomitant decline in glutathione ($p < 0.001$), ascorbic acid ($p < 0.01$) and glycogen ($p < 0.01$). Co-administration of the extract with DEHP significantly prevented the rise in the levels of lipid peroxidation ($p < 0.01$) and restored the levels of glutathione, ascorbic acid and glycogen (Table 2).

Table 2. Effect on hepatic glycogen, lipid peroxidation (TBARS) and antioxidant parameters of rats.

Treatment	Lipid peroxidation (TBARS) n moles/mg tissue	Glutathione (GSH) µ moles /g tissue	Ascorbic acid (mg/g) tissue	Glycogen (mg/gm)
Control (vehicle)	2.52 ± 0.05	3.40 ± 0.11	1.24 ± 0.07	6.01 ± 0.0.23
DEHP (1000 mg/kg b.wt.)	7.84 ± 0.11***	2.58 ± 0.12***	0.86 ± 0.05**	5.45 ± 0.11*
DEHP + *A. graveolens* extract (300 mg/kg b.wt.)	3.10 ± 0.07[b]	3.18 ± 0.12[a]	1.16 ± 0.07[a]	5.83 ± 0.27

Values are Mean±SEM
Levels of significance * p < 0.05, ** p<0.01; *** p < 0.001 when compared with control rats.
a p < 0.01; b p<0.001 when compared with DEHP treated rats.

DISCUSSION

Subchronic administration of DEHP (1000 mg/kg b.wt. /day) for 6 weeks in rats caused a significant increase in the relative liver weight. These results are in concurrence with earlier reports and can be attributed to increased fatty acid and phospholipid synthesis and enlargement of liver due to increase in cell proliferation and a decrease in apoptosis (Sakurai et al., 1978; Yanagita et al., 1987; Rusyn et al., 2006). Administration of extract significantly prevent the increase in relative liver weight suggesting a favorable modulatory influence on liver lipid metabolism.

DEHP caused a significant decrease in glycogen content of liver, which corroborates the findings of pre-vious workers who have reported a similar decrease in serum insulin and liver glycogen and an increase in blood glucose level (Sakurai et al., 1978; Gayathri et al., 2004). However the extract treatment reversed these changes probably by enhancing glucose uptake in the liver.

Assessment of liver function can be made by estimateing the activities of marker parameters like SGOT, SGPT and ALP, which are originally present in higher concentration in cytoplasm. When there is hepatocellular damage these enzymes leak in the blood circulation (Sallie et al., 1991). Elevated levels of these marker enzymes in serum of DEHP treated rats correspond to extensive liver damage. Hyperbilirubinemia observed in DEHP treated rats indicates impairment of glucuronyltransferase system leading to inhibition of bilirubin elimination (Sjoberg et al., 1991). Co-administration of methanolic extract of *A. graveolens* seeds along with DEHP significantly prevent the rise in these serum marker parameters and restored them towards normal side. These results are in agree-ment with previous findings, which have also reported significant prevention in the rise of serum, SGOT, SGPT, ALP, and bilirubin levels by A. *graveolens* extract in rats treated with various hepatotoxin supporting the anti-hepa-totoxic action of the extract by virtue of membrane stabi-lizing activity (Singh and Handa, 1995; Ahmed et al., 2002). It probably does so by free radical scavenging which has been established in the *in vitro* studies indicating its antioxidant activity (Popovic et al., 2006).

In the present investigation a significant increase in lipid peroxidation (TBARS) and a concomitant decline in liver glutathione (GSH) and ascorbic acid concentration, was observed in DEHP treated rats. Similar enhance-ment in lipid peroxidation and decline in antioxidants de-fense system after DEHP treatment in animals were observed by earlier workers (Santhosh et al., 1998; Popovic et al., 2006) . Co-administration of extract signify-cantly prevented the rise in TBARS level with a concomi-tant elevation in the concentration of hepatic glutathione and ascorbic acid suggesting alleviation of oxidative stress and restoration of antioxidant defense system resulting in membrane stabilization. Similar ameliorative effects were observed by administration of vitamin E and C along with DEHP in rats (Ishihara et al., 2000).

Co-administration of methanolic extract of A. *graveolens* seeds along with DEHP significantly prevented the rise in serum marker parameters and restored them towards normal value. It also caused significant decline in LPO and a significant elevation in the concentration of hepatic glutathione and ascorbic acid. These results are in agreement with previous findings, which have also reported significant prevention in the rise of serum SGOT, SGPT, ALP and bilirubin levels by A. *graveolens* extract in rats treated with various hepatotoxin supporting anti-hepatotoxic action of extract by virtue of membrane stabilizing activity (Singh and Handa, 1995; Ahmed et al., 2002). Similar alteration in oxidative stress markers has been observed in rat testes treated with *A. graveolens* extract (Hamza and Amin, 2007).

A. *graveolens* seeds are a rich source of phenolic constituents such as flavanoids, anthrons, xanthons and tannins. One of these Apigenin which is a flavone sub-class of flavanoid, has been estimated and found to be major fraction of *A. graveolens.* It has been found to prevents LPO elevation and protect antioxidant system in N-nitrosodiethylamine (DEN) induced and phenobarbital promoted hepatocellular carcinogenesis (Singh et al., 2004). Besides these, it also contains -tocopherol and glucosides (Ching and Mohammed, 2001; Kitajima et al., 2003). These phytoconstituents have antioxidant effect and cyclooxygenase inhibiting activity (Momin and Nair, 2001b; Sultana et al., 2005). Results obtained from this study suggest that the methanolic extract of A. g*raveolens* seeds posseses hepatoprotective activity. A further study is warranted to isolate the active principles to esta-

blish its mechanism of action.

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