Protective effect of Nigella sativa against diethyl phthalate-induced changes in mitochondrial enzymatic activities in liver of mice

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Abstract: Present study focuses on the evaluation of Diethyl phthalate (DEP) exerted hepatotoxicity in mice by measuring mitochondrial activities (Succinate dehydrogenase, Adenosine triphosphatase and phosphorylase) and its alleviation by Nigella sativa seed extract. Colony inbred Swiss strain adult female albino mice were orally administered with 310, 620 and 1240 mg/kg body weight/day (low, mid, high dose respectively) for 30 days. Treatment caused, as compared with the control, significant (p < 0.05) and dose dependent decrease of SDH, ATPase and Phosphorylase activities. Nigella sativa seed extract (150 and 300 mg/kg body weight/day) treatment along with HD of DEP, caused significant (p < 0.05) restoration in mitochondrial activities in liver as compared to DEP alone treated mice. It is concluded from the present study that supplementation of Nigella sativa extract can be beneficial in positively modulating DEP induced alterations in liver.

Key Words: Diethyl phthalate, Nigella sativa, Succinate dehydrogenase, Adenosine triphosphatase, liver, Phosphorylase.

1. INTRODUCTION:

Animal including humans beings are regularly exposed to toxic chemicals through food, water, air or from direct contact with a variety of consumer products. Many of these chemicals are toxic at some dose and under certain conditions of exposure. Through various route of exposure can significantly influence a chemical’s toxicity. Diethyl phthalate is a member of esters of phthalatic acid known as phthalates, used ubiquitously as solvents and plasticisers worldwide (ECB (European Chemicals Bureau) substance ID (2006) and Godwin et al.,(2010)). Its release into the environment occurs primarily as a result of production, use and disposal of products containing DEP. (Giam et al., (1987) and Joblins et al., (1995)). Diethyl Phthalate was found to be one of the more toxic phthalates (hauser et al.,2005). DEP, an endocrine disrupter chemical, has been found to have diverse acute and chronic toxic effects in several species at different trophic levels (Staples et al.,2000). It caused mitochondrial swelling, focal dilation and vesiculation of smooth endoplasmic reticulum and increased interstitial macrophage activity associated with the surface of Leyding cells of rats (Zou et al., 1997).

Medicinal plants are also used in the preparation of herbal medicines as they are considered to be safe as compared to modern allopathic medicines. Amongst the various medicinal plants, Nigella sativa (family Ranunalaceaes) was selected to evaluate its potency in ameliorating the DEP – induced toxicity. Nigella sativa seeds contain other ingredients, including nutritional components such as carbohydrates, fats, vitamins, mineral elements, and proteins, including eight of the nine essential amino acids (Bhatia et al., 1972; Correa et al., 1986; Jassir et al., 1992; Omar et al., 1999; Chun et al., 2002).

Therefore, the aim of the present study was investigate the possible protective effect of Nigella sativa seed extract against DEP – induced toxicity in mice.

2. MATERIALS AND METHODS:

Chemicals: Diethyl phthalate was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India and was of analytical grade. All the other chemicals used in the present study were of analytical grade and purchased from Hi Media Laboratories Pvt. Ltd., Mumbai, India, Sisco Research Laboratories Pvt. Ltd., Mumbai, India and Sigma Aldrich, St. Louis, MO, USA. Olive oil was obtained from Figaro, Madrid, Spain.

Experimental animals: All animal studies were sanctioned by Institutional Animal Ethics Committee of Gujarat University, Ahmedabad and approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India. Healthy young female albino mice of Swiss strain weighing 30-35 gm were obtained from Zydus Research Centre, Ahmedabad, India. The animals were kept in the Animal House of Zoology Department of...
Nigella sativa extract preparation: Seeds of Nigella sativa were purchased from local market and hydro-alcoholic extract was prepared according to Bhargava and Singh with slight modification. Finely ground Nigella sativa seeds powder was mixed with 50% methanol and allowed to stand overnight for maximum extraction of polyphenols. Percolation of the extract was performed at room temperature in two stages. Collected filtrate was evaporated below 50°C to obtain a final product in the form of residues which was stored under refrigerated conditions. Extract was dissolved in double distilled water and used for studies.

Experimental Design: Eighty animals were randomly divided into eight groups. Animals of Group 1 were without any treatment. Animals of Group 2 received 0.2ml olive oil/animal/day (olive oil was used to dissolved DEP) for 30 days and marked as vehicle control. Antidote control group (Group 3) animals were given oral treatment of Nigella sativa (300 mg/kg body weight/day). Group 4, 5 and 6 animals were given oral treatment of low dose (310 mg/kg body weight /day), mid dose (620 mg/kg body weight /day), and high dose (1240 mg/kg body weight/ day) of DEP. Animals of Group 7 and 8 were treated with DEP (1240 mg/kg body weight/ day) along with 150 and 300 mg/kg body weight/ day of Nigella sativa extract. Dosages of DEP treatment were based on the LD50 value i.e. 8600 mg/kg (National Toxicology Program, 2006). Animals were given treatment for 30 days and autopsied on 31st day. Liver was quickly isolated, blotted free of blood and used for determination of biochemical parameters.

Adenosine triphosphatase activity: The adenosine triphosphatase (ATPase) activity in the liver and kidney was assayed by the method of Quinn and White (1968). ATPase causes hydrolysis of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and inorganic phosphate (i.p.). The liberated inorganic phosphate was estimated by the method of Fiske and Subbarow (1925). The optical density was read at 660 nm. The enzyme activity was expressed as µmoles inorganic phosphate released/mg protein/30 min.

Succinic dehydrogenase activity: The succinic dehydrogenase (SDH) activity in the liver and kidney was assayed by the method of Beatty et al. (1966) using 2-(4-iiodophenyl)-3-(4-nitrophenyl)-5- phenyl- 2H – tetrazolium chloride (INT) as an electron acceptor. The electrons released by the enzyme SDH from the substrate are taken up by INT, which was reduced to a red coloured formazan. This was extracted in ethyl acetate and the absorbance was read at 420 nm. The enzyme activity was expressed as µg formazan formed/mg protein/15 min.

Phosphorylase activity: The liver phosphorylase activity was assayed by the method of Cori et al. (1943). The inorganic phosphate (i.p.) formed at the end of the reactions was estimated by the method of Fiske and Subbarow (1925). The enzyme phosphorylase hydrolyses the substrate glucose -1-phosphate. The inorganic phosphate formed at the end was treated with an acidic molybdate solution; it forms phosphomolybdic acid which on addition of 1-amino-2-nepthol-4-sulphonic acid (ANSA) is quantitatively reduced to a blue coloured complex which is measured spectrophotometrically at 660 nm. The enzyme activity is expressed as µg phosphorus released/100 mg fresh tissue/15 min.

Hepatoprotective index (HP index): Hepatoprotective index (HP index) The liver protecting activity of the Nigella sativa seed extract was expressed as hepatoprotective percentage (H) (Prakash et al. 2008) which was calculated using the formula:

\[ H = \left(1 - \frac{T - V}{C - V}\right) \times 100 \]

Where T is the mean value of plant extracts along with the DEP, C is the mean value of DEP alone, and V is the mean value of vehicle control animals.

Statistical analysis: All the data are expressed as the means ± standard error mean (SEM). Statistical analysis was performed using Graphpad Instat, software, version 5.03. The data were statistically analyzed using one - way Analysis of Variance (ANOVA) followed by Tukey’s test. The level of significance was accepted with p < 0.05.
3. RESULT AND DISCUSSION:

Table 1: Showing effect of diethyl phthalate on succinic dehydrogenase, adenosine triphosphatase and phosphorylase activities in liver of mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental groups</th>
<th>Untreated 1</th>
<th>Vehicle control 2</th>
<th>Low dose of DEP 3</th>
<th>Mid dose of DEP 4</th>
<th>High dose of DEP 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDH</td>
<td></td>
<td>55.87 ± 2.03</td>
<td>54.29 ± 1.92</td>
<td>41.60 ± 1.12 a</td>
<td>31.12 ± 1.01 a</td>
<td>26.44 ± 0.76 a</td>
</tr>
<tr>
<td>ATPase</td>
<td></td>
<td>1.51 ± 0.06</td>
<td>1.54 ± 0.03</td>
<td>1.34 ± 0.07</td>
<td>0.92 ± 0.12 a</td>
<td>0.50 ± 0.02 a</td>
</tr>
<tr>
<td>Phosphorylase</td>
<td></td>
<td>1.39 ± 0.04</td>
<td>1.37 ± 0.02</td>
<td>1.15 ± 0.02 a</td>
<td>0.89 ± 0.04 a</td>
<td>0.52 ± 0.05 a</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M.; n = 10
Significant at the level
*p < 0.05 verses vehicle control group (Group 2)
No significant difference between control groups (Group 1 and 2)
Units: SDH - µg formazion formed/mg protein/15 min; ATPase - µmoles i.p. released/mg protein/30 min; phosphorylase - µg phosphorus released/100 mg fresh tissue/15 min.

Table: 1 depicts the results of various doses of DEP caused changes in energy metabolism. No significant changes were observed in the activities of SDH, ATPase and phosphorylase in the liver of untreated and vehicle control group (group 1 and 2). However, in all three doses of DEP treatment (Group 3, 4 and 5) significant (p<0.05) reduction was observed in the activity of SDH (LD: 23.38%, MD: 42.68%, HD: 51.30%). However, significant reduction was in mid and high doses DEP-treated groups. The reduction in both SDH and ATPase activities were dose-dependent (r² =0.963 and 0.977 respectively). Similarly, oral administration of three different doses (LD, MD and HD) of DEP caused decrease phosphorylase activity significantly (p<0.05) and dose – dependently (r² = 0.985) in liver (16.06%, 35.04% and 62.05% respectively).

Oral administration of DEP to mice for 30 days had significantly altered the energy status. DEP treatment resulted in reduction in SDH activity – an enzyme bound to inner mitochondrial membrane, which could be due to structural and functional disorganization of the mitochondrial assembly. Srivastava et al. (1978 and 1977) reported that di (2-ethyl hexyl) phthalate (DEHP) also found to inhibit the activity of total and Mg+ - stimulated ATPase activity in rat liver. Beside liver, the activity of SDH and ATP se was also inhibited in rat heart, kidney (Srivastava et al., 1977), lung and gonads (Seth et al., 1976), indicating that suppression of energy- linked reactions may be a generalized effect of DEHP. Alteration in mitochondrial potential decreases the rate of cellular ATP synthesis and, thus nucleotide synthesis which may cause the reduction in DNA and RNA contents. Energy deficiency of the cell characterised by reduced activity of SDH and ATPase could be well correlated with reduction in protein content (Panet and Altan, 1979).

Mitochondria contains biochemical machinery for oxidation of various biomolecules and produced energy is captured in the form of ATP. Phthalates inhibited the respiration of isolated mitochondria from rat liver primarily by uncoupling oxidative phosphorylation (Inouye et al., 1978; Melnick et al., 1982). Other researchers have suggested that the phthalates inhibited electron transport or energy transport (Ohyama et al., 1976). Dibutyl phthalate and dimethyl phthalate inhibited the activities of SDH and ATPase, enzymes of the rat liver inner mitochondrial membrane (Srivastava et al., 1977; Tanaka et al., 1978; Melnick et al., 1982).

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>SDH</th>
<th>ATPase</th>
<th>Phosphorylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Vehicle</td>
<td>54.29 ± 1.92</td>
<td>1.54 ± 0.03</td>
<td>1.37 ± 0.02</td>
</tr>
<tr>
<td>2. Antidotet(NS300)</td>
<td>54.19 ± 1.78</td>
<td>1.60 ± 0.05</td>
<td>1.39 ± 0.04</td>
</tr>
<tr>
<td>(II) Diethyl phthalate – Treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. DEP1240 ; HD</td>
<td>26.44 ± 0.76 a</td>
<td>0.50 ± 0.02 a</td>
<td>0.52 ± 0.05</td>
</tr>
<tr>
<td>(IIID) DEP1240(HD)+ Nigella sativa extract – Treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. HD DEP + NS150</td>
<td>35.06 ± 1.56 a</td>
<td>0.78 ± 0.02 a</td>
<td>0.83 ± 0.02 ab (37.00)</td>
</tr>
<tr>
<td></td>
<td>(31.00)</td>
<td>(27.00)</td>
<td></td>
</tr>
<tr>
<td>5. HD DEP + NS300</td>
<td>49.27 ± 2.45 b</td>
<td>1.07 ± 0.06 ab (58.50)</td>
<td>1.0 ± 0.02 ab (57.00)</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M.; n = 10
Significant at the level
*p < 0.05 versus vehicle control group (Group 2)
*p < 0.05 versus high dose DEP - treated group (Group 3)
No significant difference between control groups (Group 1 and 2)

Values in parenthesis indicate hepatoprotective index (HPI)

Units: SDH - µg formazone formed/mg protein/15 min; ATPase - µmoles i.p. released/mg protein/30 min

Oral administration of 300 mg/kg bw *Nigella sativa* seed extract did not cause any significant change than that of vehicle control (Group 2) (Table 2). High dose of DEP treatment reduced activities of hepatic SDH, ATPase and phosphorylase to 48.70% and 32.46% respectively as compared to vehicle control. Cotreatment of *Nigella sativa* seed extract along with HD of DEP for 30 days caused significant amelioration in all parameters as compared to HD of DEP alone treated groups (Table: 2). Percent protection in SDH and ATPase activities were NS150: 31.00, NS300: 82.00 and NS150: 27.00, NS300: 58.50 respectively as indicated by HPI. Cotreatment of *Nigella sativa* seed extract along with high dose of DEP for 30 days caused significant amelioration in phosphorylase activity as compared to HD of DEP alone treated groups. Percent protection in phosphorylase activity was NS150: 37.00, NS300: 57.00 as indicated by HPI. All three doses of DEP were found to reduce the activities of SDH and ATPase activities in liver of the animals resulting in altered status. Treatment with *Nigella sativa* seed extract along with DEP significantly ameliorates DEP caused changes in the activities of SDH and ATPase in liver and kidney of mice (Table 1). Erşahin et al. (2011) reported that *Nigella sativa* with its potent free radical scavenging properties, inhibited subarachnoid-haemorrhage-(SAH-) induced lipid peroxidation in the brain tissue of rat against the reactive hydroxyl, peroxyl, and superoxide radicals. In addition, the level of antioxidant glutathione (GSH) was preserved, thereby ameliorating oxidative damage. The SAH-induced reduction of Na+ /K+ -ATPase activity indicated the presence of membrane damage. The Na+/K+/ ATPase is involved in the generation of the membrane potential through the active transport of sodium and potassium ions in cellular membrane. It maintains neuronal excitability and controls cellular volume in the central nervous system. Treatment with *Nigella sativa* was able to restore Na+ /K+ -ATPase activity back to normal levels. Hamed et al. (2013) reported that treatment with black seed alleviated the elevation of SDH and Na+/K+ + ATPase. The restoration of ATPase activity suggest the ability of *Nigella sativa* to protect the sulphydryl group from oxidative damage through inhibition of lipid peroxidation. Normalised metabolism of protein, carbohydrates and lipid as well as free radical scavenging effect of plant improves integrity and oxidative phosphorylation in mitochondria which was highly disturbed in case of energy deficient state-induced by DEP.

4. CONCLUSION:

In conclusion, oral administration of DEP caused alteration in Succinate dehydrogenase, Adenosine triphosphatase and phosphorylase activities in liver. *Nigella sativa* seed extract reduced DEP induced mitochondrial enzymatic changes due to its phytochemicals having antioxidative properties.

REFERENCES: