Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 3| Issue 3 |JUL-SEP|2013|443-449

Research Article
Biological Sciences

NIGELLA SATIVA AMELIORATES DIETHYL PHTHALATE -INDUCED LIPID INFILTRATION IN LIVER OF MICE

Heena Prajapati And Ramtej J. Verma*

Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad - 380009, India

***Corresponding Author Email:** ramtejverma2000@yahoo.com

ABSTRACT

Nigella sativa is a widely used medicinal plant throughout the world. The present investigation was an attempt to evaluate the mitigatory effect of Nigella sativa seed extract on diethyl phthalate (DEP) induced liver damage. Swiss strain adult female albino mice were orally administered with 310, 620 and 1240 mg/kg body weight/day DEP with or without Nigella sativa extract (150 and 300 mg/kg body weight/day) for 30 days. Results revealed that oral administration of DEP caused significant dose-dependent increase in cholesterol and total lipid contents while protein content decreased in liver of mice as compared with vehicle control. However, cholesterol content significantly decreased in serum of toxin treated mice. Histopathological studies revealed fat deposition, intra – cellular vacuolation, necrosis and loss of hepatic architecture in DEP – treated animals. Co- treatment of Nigella sativa seed extract (150 and 300 mg/kg body weight) along with DEP (HD) alleviates the toxin induced changes in cholesterol, protein and total lipid contents as well as histoarchitecture of liver in mice. The effect was dose-sependent. 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, lipid infiltration

INTRODUCTION

Diethyl phthalate is a member of esters of phthalatic acid known as phthalates, used ubiquitously as solvents and plasticisers worldwide [1 and 2]. Its release into the environment occurs primarily as a result of production, use and disposal of products containing DEP [3 and 4]. Also, DEP may enter atmosphere through combustion of plastics and to a lesser degree by volatilization [5]. In addition, DEP is widely used in the perfume binder industry as a vehicle for fragrances and in personal care products making human exposure of DEP significant to adults as well as neonatal, as confirmed by level recorded in blood as well as breast milk samples of human populations in some parts of the world [6]. DEP is known to cause oxidative stress [7].

World is endowed with a wealth of medicinal plants which are rich sources of pharmacologically active phytochemicals possessing numerous therapeutic properties. Medicinal plants are also used in the preparation of herbal medicines as they are considered to be safe as compared to modern allopathic medicines. Among the various medicinal plants, *Nigella sativa* (family Ranunalaceaes) is commonly known as black seed was selected to evaluate its ameliorative effect against the DEP – induced toxicity. *Nigella sativa* has been used traditionally, especially in the Middle East and India, for the treatment of asthma, cough, bronchitis, headache, rheumatism, fever, influenza and eczema [8]. Recently concluded clinical and experimental researches have shown many therapeutic effects of *Nigella sativa* such as immunomodulator [9], anti-inflammatory [10] and anti-tumor agents [11].

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

MATERIALS AND METHODS

Chemicals

Analytical grade diethyl phthalate (DEP) (CAS No. 84-66-2) was purchased from Sisco Research Laboratories, Mumbai, India. All other chemicals used in the present study were of analytical grade.

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 [12]. 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 animals

Swiss strain colony bred healthy young female albino mice (*Mus musculus*) weighing 30-35 gm were obtained from Zydus Research Centre, Ahmedabad, India. The animals were kept in the Animal House of Zoology Department of Gujarat University, Ahmedabad, India under controlled conditions (Temperature 25±2˚C, 12h light/dark cycle and relative humidity 50-55%). They were fed with certified pelleted rodent feed supplied by Amrut Feeds, Pranav Agro Industries Ltd., Pune, India and water *ad libitum*. All the experimental protocols were approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg-167/1999/CPCSEA), New Delhi, India. Animals were handled according to the guidelines published by the Indian National Science Academy, New Delhi, India (1991).

Experimental design and treatment schedule:

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 LD_{50} value i.e. 8600 mg/kg [13].

Animals were given treatment for 30 days and autopsied on 31st day. Blood samples collected by cardiac puncture in non - anticoagulant added tubes were allowed to clot and centrifuged at 1000 x g for 10 min at 4˚C. Non – haemolysed serum samples were stored at -4˚C and used for biochemical analysis. Liver was quickly isolated, blotted free of blood and used for determination of biochemical parameters.

Total lipid content

Total lipid content in the liver was estimated according to the method of Fringes et al. [14] using olive oil as a standard. Lipid on being heated with sulphuric acid followed by addition of vanillin and phosphoric acid produces a pink colour whose optical density was measured at 530 nm. The total lipid content was expressed as mg/100 mg tissue weight.

Total cholesterol content

The concentration of cholesterol was estimated in the liver and serum by the method of Zlatki et al. [15]. Cholesterol forms a coloured complex with $FeCl_3$ in the presence of concentrated sulphuric acid and glacial acetic which can be measured at 540 nm. The cholesterol content was expressed as mg/100 mg tissue weight for liver and mg/dL for serum.

Protein content

Protein content was measured in liver by the method of Lowry *et al*. [16] using bovine serum albumin as a standard. The protein content was expressed as mg/100 mg tissue weight.

Histopathology

Histopathological studies were carried out using the standard technique of hematoxylin and eosin

staining. The fresh pieces of liver were fixed for 18 h in alcoholic Bouin's fixative. The tissue was dehydrated by passing through ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. 5 µm thick sections were cut on a rotary microtome and stained with Ehrlich's hematoxylin - eosin (alcohol soluble), dehydrated in alcohol, cleared in xylene, mounted in DPX and examined microscopically.

Hepatoprotective index (HP index)

The liver protecting activity of the *Nigella sativa* seed extract was expressed as hepatoprotective percentage (H) [17] which was calculated using the formula:

$$H = 1 - \frac{(T - V)}{(C - V)} \times 100$$

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 anwas 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 $p < 0.05$.

RESULTS

Lipid, cholesterol and protein contents in liver of animals treated with *Nigella sativa* alone (Group 3) remained within normal levels as that of the control groups (Group 1 and 2).

Oral administration of DEP for 30 days caused, as compared with the vehicle control, significant ($p<0.05$) and dose-dependent increase in cholesterol and lipid contents. In addition, treatment caused significant ($p<0.05$) dose-dependent decrease in protein content. However, the cholesterol content was significantly decreased in serum of DEP treated group of mice as compared to vehicle control.

As compared with high dose of DEP, co-treatment with *Nigella sativa* seed extract along with high dose DEP caused significant amelioration in total lipid, cholesterol and protein contents in liver of mice. The effect was dose-dependent. Hepatoprotective index calculated for total lipid [34.70% (NS 150) and 70.00% (NS 300)], cholesterol [48.00% (NS150), 70.00% (NS 300)] and protein [29.00% (NS 150) and 61.00% (NS300)] were significantly higher.

Cotreatment of *Nigella sativa* seed extract along with high dose of DEP caused significant reverses in the cholesterol level in serum [HP index = 36.00% (NS150), 54.00% (NS300)] as compared to the DEP alone treated mice.

The transverse section of liver of vehicle control (**Plate 1**) and antidote control (**Plate 2**) mice showed a normal histo-architecture. Oral administration with high dose (1240 mg/kg body weight/day) of DEP for 30 days caused severe fat deposition, intra – cellular vacuolation, necrosis and loss of hepatic architecture as compared to control (**Plate 3**). Co-treatment with *Nigella sativa* seed extract (150 and 300 mg/kg body weight/day) along with DEP caused almost complete amelioration as fat deposition, hepatocellular necrosis, intracellular vacuolation were almost absent (**Plate 4 and 5**).

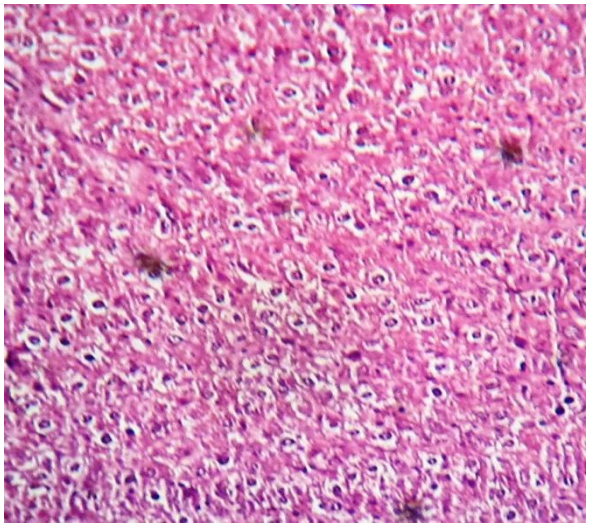

Plate 1: Histopathology of vehicle control mice, normal histo-architecture (100X).

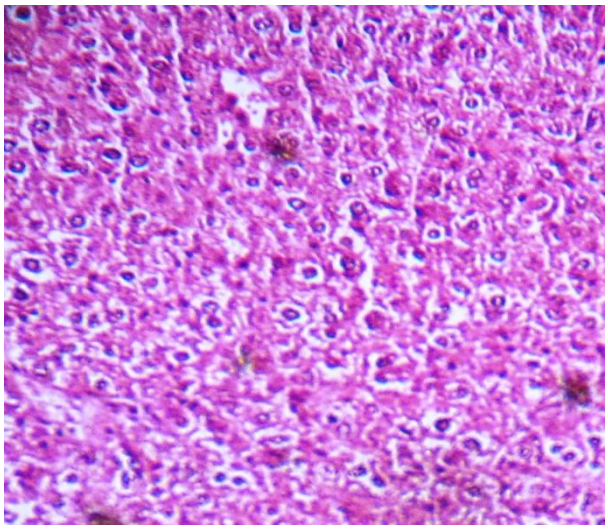

Plate 2: Histopathology of antidote control mice, normal histo – architecture (100X)

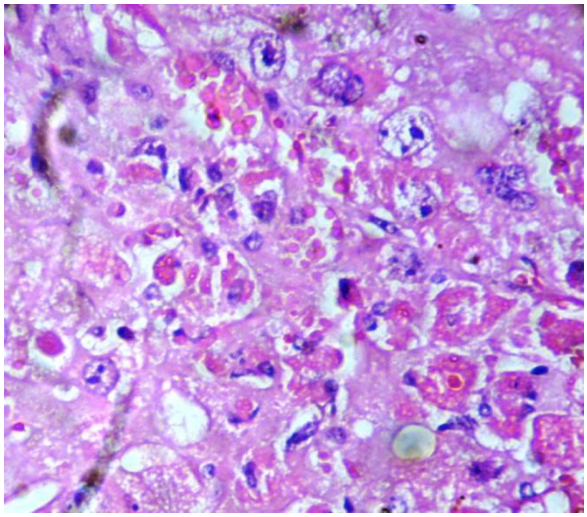

Plate 3: Histology of HD – diethyl phthalate – treated mice, severe fat deposition intracellular vacuolation and loss of hepatic architecture and necrosis (100x).

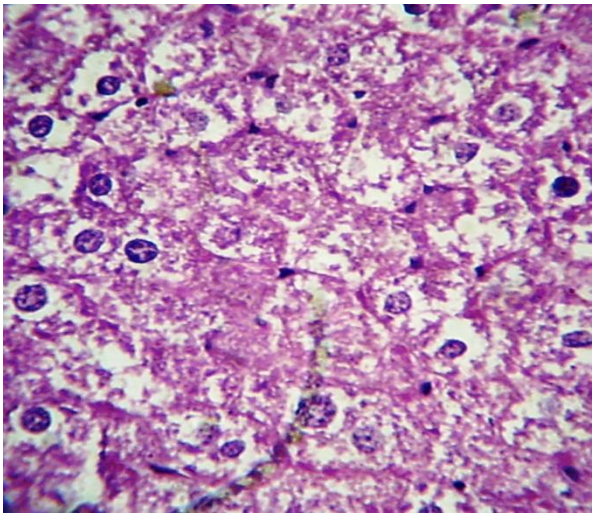

Plate 4: Histology of HD – diethyl phthalate along with 150 mg *Nigella sativa* seeds extract – treated mice, fat deposition, intracellular vacuolations, and necrosis absent (100 x).

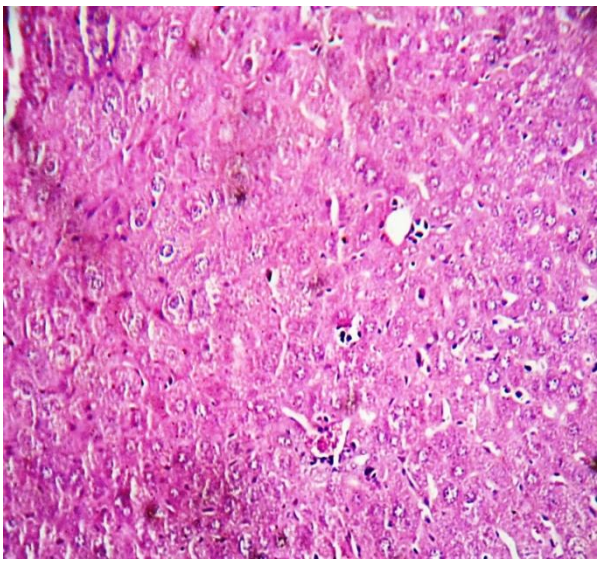

Plate 5: Histology of HD – diethyl phthalate along with 150 mg *Nigella sativa* seeds extract – treated mice, normal hepatic architecture and almost complete recovery (100x).

DISCUSSION

The present study clearly indicates that oral administration of DEP for 30 days caused increase in cholesterol and total lipid content in liver as well as significant reduction in serum cholesterol content. Many investigators [18 and 19] have reported increased level of cholesterol in liver as well as its decrease in serum of mice and rat. Histopathological studies also revealed significant deposition of fat in liver of DEP treated mice. In addition, our earlier study revealed significant increase in absolute and relative weight which could be due to fat deposition in liver [7].

DEP treatment caused significant reduction in protein content in liver of mice. Prajapati and Verma [7] have reported DEP induced lipid peroxidation in liver of mice. Sun et al [20] also reported oxidative stress caused by DEP.

Nigella sativa seed extract ameliorated DEP induced changes in lipid, cholesterol and protein contents as well as histopathological changes in liver of mice. It could be due to its antioxidative activity [7]. The antioxidant effect of *Nigella sativa* seed seems to be due to thymoquinone, flavonoids and also antioxidant vitamins like ascorbic acid. Flavonoids are a class of polyphenolic compounds that seem to

have antioxidative properties by suppressing reactive oxygen and nitrogen species formation, scavenging reactive oxygen and nitrogen species and protecting the antioxidant defense system [21 and 22]. *Nigella sativa* treatment returned total protein content to near normal was reported by Ayed et al [23]. *Nigella sativa* has been found to lower the doxorubicin and CCL_4 – induced up-regulation of cholesterol in liver tissue [24 and 25].

CONCLUSION

In conclusion, oral administration of DEP caused alteration in lipid, cholesterol and protein contents as well as histopathology in liver, which could be a principal mechanism responsible for its hepatotoxicity. *Nigella sativa* seed extract reduced DEP induced hepatic changes due to its phytochemicals having antioxidative properties.

REFERENCES

1. ECB (European Chemicals Bureau) substance ID: 84-66-2. Diethyl phthalate IUCLID dataset. European chemical bureau. European commission. 35pp. Available online at:

   http://www.epa.gov/hpv/pubs/summaries/benzene/cl3467rr3dd.pdf, (2006)
2. Godwin A., Uses of phthalates and other plasticizers. Exxonmobil chemical company, (2010)
3. Giam CS, Wong MK., Plasticizers in food. J. Food Prot, 50:769-782,(1987)
4. Joblins S, Reynolds T, White R, Parker MG, Sumpter JP., A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ. Health Perspect, 103:582-587, (1995)
5. US EPA. International Risk Information System: Diethyl phthalate (CASRN: 84-66-2). Whshington, DC, US Environmental Protection Agency, at website http://www.epa.gov/iris/subst/0266.htm, (1993)
6. Pereira C, Mapuskar K, Rao CV., chronic toxicity of diethyl phthalate – A three generation lactational and gestational exposure study on male wistar rats. Environ. Toxicol. Pharmacol., 23:319-327, (2007)
7. Prajapati H, Verma RJ., Mitigation of diethyl phthalate –induced hepatotoxicity by Nigella sativa seed extract in mice. Int. J. Pharm. Bio Sci, 4(4):(B) 1366-1376, (2013)
8. Burits M, Bucar F., Antioxidant activity of Nigella sativa essential oil, Phytother Res, 14:323-328, (2000)
9. Houghton PJ, Zarka R, de las Heras B, Hoult JR. Fixed oil of Nigella sativa and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta. Med. 61: 33-36, (1995).
10. El Daly ES. Protective effect of cysteine and vitamin E, Crocus sativus and Nigella sativa extracts on cisplatin-induced toxicity in rats. J. Pharm. Belg. 53: 87-93; discussion 93-95, (1998).
11. Nagi MN, Mansour MA. Protective effect of thymoquinone against doxorubicin – induced cardiotoxicity in rats: a possible mechanism of protection. Pharmacol. Res. 41: 283 – 289, (2000).
12. Bhargava K.P., Singh N. Antistress activity of *Ocimum scnctum* Linn. Indian J. Med. Res. 73:443, (1981).
13. NTP, Chemical information profile for diethyl phthalate. National Toxicology Program, (2006).
14. Fringes Cs, Fendly TW, Dunn RT and Queen CA., Improved determination of total serum lipids by the sulpho vanillin reaction. Cin. Chem., 18:673-674, (1972)
15. Zlatki A, Zak B and Boyle GJ., A new method for the determination of serum cholesterol. J. Lab. Clin. Med., 41:486, (1953)
16. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ., Protein measurement with the folin phenol reagent. J. Biochem. 4: 193-265, (1951)
17. Prakash T, Fadadu SD, Sharma UR, Surendra V, Goli D,Stamina P and Kotresha D., Hepatoprotective activity of leaves of Rhododendron arboreum in CCl4 induced hepatotoxicity in rats. J. Med. Plants Res. 2(11) : 315-320, (2008)
18. Mapuskar K, Pereira C, Rao CV., Dose- dependent sub-chronic toxicity od diethyl phthalate in female swiss mice. Pest. Biochem. Physiol. 87:156-163, (2006)
19. Pereira C, Mapuskar K, Rao CV., Chronic toxicity of diethyl phthalate in male wistar rats-A dose-dependent study. Regul. Toxicol. Pharmacol. 45:169-177, (2006)
20. Sun Y, Takahashi K and Hosokawa T *et al*. Diethyl phthalate enhances apoptosis induced by serum deprivation in PC 12 cells. Basic Clin. Pharmacol. Toxicol. 111(2): 113-119, (2012)
21. Arts IC, Hollman PC., Polyphenols and disease risk in epidemiologic studies. Am. J. Clin. Nutr. 81: 317S – 325S, (2005)
22. Moyers SB, Kumar NB., Green tea polyphenols and cancer chemoprevention: multiple mechanisms and endpoints for parts II trials. Nutr. Rev. 62 (5): 204 – 211, (2004)

23. Ayed Sh AL- LOGMANI and Talal A ZARI., Effect of Nigella sativa L. And cinnamomum zeylanicum blume oils on some physiological paramerers in streptozotocin-induced diabetic rats. Boletin Latinoamericano Y Del Caribe de plantas Medicinales Y Aromaticas., 8(2): 86-96, (2009)
24. Badary OA, Abdel-Naim AB, Abdel-Wahab MH, Hamada FM., The influence of thymoquinone on doxorubicin-induced hyperlipidemic nephropathy in rats. Toxicol. 143:219-226, (2000)
25. El- Dakhakhny M, Mady NI, Halim MA., Nigella sativa L., Nigella sativa L. oil protects against induced hepatotoxicity and improves serum lipid profile in rats. Arzneimittelforschung. 50:832-836, (2000)

***Corresponding Author:**
Ramtej J.Verma*
Department of Zoology,
School of Sciences,
Gujarat University,
Ahmedabad – 380009, India.
E-mail: ramtejverma2000@yahoo.com,
patelmonalir@gmail.com