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EFFECT OF *N*- NITROSODIETHYLAMINE ADMINISTRATION ON LIVER OF WISTAR RATS

Joice P. Joseph[1], S.K. Raval[1], D.J. Ghodasara[2], Jayesh Patel[3], Vihol Karan Singh[3] and Bhavisha Jivani[2]

[1]Department of Veterinary Medicine, College of Veterinary Science & Animal Husbandry, Anand Agricultural University, Anand-388 001, Gujarat, India

[2]Department of Veterinary Pathology, College of Veterinary Science & Animal Husbandry, Anand Agricultural University, Anand-388 001, Gujarat, India

[3]Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science & Animal Husbandry, Anand Agricultural University, Anand-388 001, Gujarat, India

Abstract: *N*- nitrosodiethylamine is a potent environmental carcinogen. Serology and histopathology of liver of wistar rats were carried out during this subchronic toxicity study. Two groups of animals were used for this experiment. *N*- nitrosodiethylamine was administered to group II animals, where as Group I animals were kept as control. Serological examination revealed development of carcinogenesis in group II animals. After experimental period, changes in morphological and histological features of liver were examined. While liver of group I animals remain normal, neoplastic changes were observed in group II animals confirming hepatocarcinogenic effect of *N*- nitrosodiethylamine.

Keywords: *N*- nitrosodiethylamine, Histomorphology, Hepatocarcinogenesis

INTRODUCTION

N- nitrosodiethylamine (NDEA) is a potent hepatocarcinogen and is a by-product of nitrosation of primary amines (from tobacco products) in the acidic conditions of stomach. It is further converted into an active ethyl radical metabolite that reacts with DNA, leading to mutations and oncogenesis (Anis *et al*., 2001). NDEA exposure can occur through diet and is detectable in edible vegetable oil, cheese, soybeans, smoked, salted and dried fish, cured meat, alcoholic beverages, cosmetics and agricultural chemicals. Metabolism of certain therapeutic drugs is also reported to produce *N*- nitrosodiethylamine. NDEA is the most important environmental carcinogen in its class that primarily induces tumours of the liver because of its relatively simple metabolic pathway and potent carcinogenic activity (Leoppky, 1994) and becomes metabolically active by the action of cytochrome p450 enzymes to produce reactive electrophiles, which increase oxidative stress level leading to cytotoxicity, mutagenecity and carcinogenicity. Commonly used antiepileptic drug, phenobarbitone is a cytochrome p450 inducer and is used in this experiment for increased metabolism of NDEA. Carcinogenicity of this chemical is studied during this experiment.

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MATERIALS AND METHODS

The experiment was carried in Wistar rats in conformity with the Institutional Animal Ethical Committee (IAEC no. 2010/Med/85), Veterinary College, Anand. Female adult rats of Wistar strain weighing 150 – 200 g were procured from Zydus Research Centre, Ahmedabad. They were housed in well ventilated polypropylene cages at air conditioning system with regulated temperature at 24^0C and humidity at 50-60% on a 12 hr day and night cycles using artificial lights. Animals were fed with commercial feed for rats and mice and distilled water *ad libitum*. Twelve animals were used for this experiment and were divided into two comprising of 6 animals in each group. Group I served as water control consisted of healthy animals. *N*- nitrosodiethylamine (Sigma Chemical Company, St. Louis, MO, USA) was administered to Group II animals at dose of 200 mg/kg mixed in saline intraperitoneally after acclimatization period of 1 week. One week after administration of *N*-nitrosodiethylamine, 0.05% of Phenobarbitone was administered to these animals by incorporating in drinking water for 13 weeks.

Serology

After experimental period**,** activity of hepatic enzymes (alanine aminotransferase (ALT)-mod. IFCC method, aspartate aminotransferase (AST)-mod. IFCC method, alkaline phosphatase (AKP)- PNPP Kinetic method, γ-glutamyl transferase (GGT)-carboxy substrate method and lactate dehydrogenase (LDH)- mod. IFCC method) and total Bilirubin-mod. Jendrassik and Grof's method were measured in serum using commercial diagnostic kits procured from Crest Biosystem (A Division of Coral Clinical System, Goa with the help of fully automatic analyser (BS-120, Mindray). Concentration of rat hepatocellular carcinoma marker alpha-2 macroglobulin (A2M) in serum of animals was also estimated using enzyme linked immunosorbent assay (ELISA) kit (Immunology Consultants Laboratory, Newberg, USA).

Histopathology

After experimental period, animals were fasted overnight and sacrificed by cervical dislocation under diethyl ether anesthesia following animal ethic guidelines. Immediately after sacrificing, livers of rats were rapidly excised and were washed in ice-cold isotonic saline, blotted to dryness and observed for the presence of gross lesions. Mean (± SE) wet liver weight of group I and group II animals were compared.

Liver samples were collected in 10% formalin. Formalin fixed tissues were processed by paraffin wax embedding method of tissue sectioning. Sections were cut at 5-6 microns

thickness and were stained with haematoxylin and eosin (H & E) stain (Luna, 1968). The H & E stained slides were observed under microscope and lesions were recorded.

Statistical analysis

The data obtained was analyzed by standard statistical procedure described by Snedecor and Cochran (1992) and were expressed as mean ± SEM (Standard error of mean).

RESULTS AND DISCUSSION

Serological Examination

Hepatic enzymes were significantly ($P < 0.05$) elevated in group II animals compared to group I (Table I). Eventhough bilirubilin concentration in group II animals were statistically nonsignificant, two animals in HCC control (group II) showed icteric plasma having bilirubin value of 2.47 mg/dl and 2.31mg/dl. These values are higher than normal range observed by Balamurugan *et al*. (2009) (0.25-0.74 mg/dl). Also tumour marker, alpha2 macroglobulin concentration was significantly ($P < 0.05$) increased in serum of group II animals (9.40 ± 1.79 ng/ml) compared to normal animals (1.07 ± 0.25 ng/ml). Results of serological studies indicate hepatic injury and occurrence of hepatocellular carcinoma in group II animals and are in accordance with findings of Premalatha *et al*., 1999; Ramakrishnan *et al*., 2007; Sadik *et al*., 2008; Shaarawy *et al*., 2009; Jahan *et al*., 2011.

Table 1. Concentration of Hepatic Enzymes in serum samples of rats in different groups

Group	ALT (U/L)	AST (U/L)	AKP (U/L)	GGT (U/L)	LDH (U/L)
I(Water control)	49.86±.59	185.51±43.46	120.46±23.08	43.04±6.05	148.87 ±6.41
II(NDEA administered)	563.54*± 42.49	389.00*±52.66	587.06*±122.72	70.64*±4.56	250.32*±14.96

* Significant at $P < 0.05$, NDEA= *N*-nitrosodiethylamine

Liver weight

Liver weight was increased in group II animals (8.45± 0.20) significantly ($P < 0.05$) compared to group I (7.11 ± 0.12). Similar results were observed by Gowthamkumar *et al.* (2010) and Kartik *et al*. (2010) also. NDEA-induced proliferation of cells in the liver tissue could be the reason for increased liver weight of Group II animals.

Gross examination of liver

Liver was examined for gross abnormalities during necropsy in all animals. In the group I, liver was found to be normal. In the group II, diffused white circular lesions of necrotic foci were present on the liver (Fig 1) of one animal. Another animal in group II showed severely pale liver.

Histological features of Liver

Histopathological examination of liver sections from control rats (group I) showed normal architecture, characterized by polyhedral shaped hepatocytes and cytoplasm granulated with small uniform nuclei (Fig 2). Hepatocytes were arranged in well-organized hepatic cords and separated by narrow blood sinusoids. In contrast, the liver sections of rats in group II showed loss of lobular architecture, necrosis, fatty changes (Fig 3), cytomegaly with karyomegaly (Fig 4) as well as vesicular active nuclei and presence of more than one nucleolus (Fig 5). Also, the nuclei of many hepatocytes appeared malignant or had the features of degenerating and dividing process. All these neoplastic changes in group II animal indicates the carcinogenic action of NDEA. Morsy *et al*. (2010) also observed fatty infiltration of hepatocytes, cytomegaly with karyomegaly as well as vesicular active nuclei and presence of more than one nucleolus in NDEA treated animals. While Kartik *et al*. (2010) reported slightly larger and more irregular hepatocytes with prominent nuclei in NDEA and carbon tetrachloride administered animals. Lesions observed in group II animals denote neoplastic changes developed by the administration of *N*- nitrosodiethylamine in liver.

All of these observations point towards one of the reasons of hepatocarcigenesis and is a warning to control environmental pollutants and to practice good food habits for better health of society.

REFERENCES

[1] Anis, K.V., Rajeshkumar, N.V. and Kuttan, R. (2001). Inhibition of chemical carcinogenesis by biberine in rats and mice. *J Pharm Pharmacol.,* **53**: 763-768.

[2] Balamurugan, M., Parthasarathi, K., Cooper, E. L. and Ranganathan, L.S. (2009). Anti-inflammatory and anti-pyretic activities of earthworm extract-*Lampito mauritii* (Kinberg). *J. Ethnopharmacol*., **121**(2): 330-332.

[3] Gowtham Kumar, S., Ramakrishnan, V., Madhusudhanan, N., Thiyagarajan, G. and Anbu selvam, C. (2010). Therapeutic efficacy of Allyl isothiocyanate evaluated on *N*-Nitrosodiethylamine/phenobarbital induced hepatocarcinogenesis in Wistar rats. *Adv. Lab. Res. Biol.,* **1**(1): 6-12.

[4] Jahan, M.S., Vani, G. and Shyamaladevi, C.S. (2011). Anti-carcinogenic Effect of *Solanum trilobatum* in Diethylnitrosamine Induced and Phenobarbital Promoted Heapatocarcinogenesis in Rats. *Asian J. Biochem.,* **6**: *74-81.*

[5] Kartik, R., Rao, C.V., Trivedi, S.P., Pushpangadan, P. and Reddy, G.D (2010). Amelioration effects against *N*-nitrosodiethylamine and CCl_4- induced hepatocarcinogenesis

in Swiss albino rats by whole plant extract of *Achyranthes aspera. Indian J. Pharmacol.,* **42** (6): 370-375.

[6] Leoppky, R.N. (1994). Nitroso Compounds Chemistry and Biochemistry. In: Nitrosamine and related compounds: Chemistry and Biochemistry, Edn., ACS, Washington DC.

[7] Luna, A.G. (1968). Manual of histological staining methods of the Armed Forces Institute of Pathology, 3rd edition. Mc Graw Hill book Co, London.

[8] Morsy, A.F.M., Ibrahim, H.S. and Shalaby, M.A. (2010). Protective effect of Broccoli and Red Cabbage against Hepatocellular Carcinoma induced by N- Nitrosodiethylamine in rats. *Am. J. Sci.,* **6**(12): 1136-1144.

[9] Premalatha, B. and Sachdanandam, P. (1999). Effect of *Semecarpus anacardium* nut extract against aflatoxin B1-induced hepatocellular carcinoma. *Fitoterapia,* **70**(5): 484-492.

[10] Ramakrishnan, G., Augustine, T. A., Jagan, S., Vinodhkumar, R. and Devaki, T. (2007). Effect of silymarin on *N*-nitrosodiethylamine induced hepatocarcinogenesis in rats. *Exp Oncol*., **29**: 39–44.

[11] Sadik, N.A.H., EL-Maraghy, S.A. and Ismail, M.F. (2008). Diethylnitrosamine-induced hepatocarcinogenesis in rats: possible chemoprevention by blueberries. *Afr. J. Biochem. Res.,* **2**(3): 081-087.

[12] Shaarawy, S.M., Tohamy. A.A., Elgendy, S.M., Elmageed, Z.Y.A., Bahnasy, A., Mohamed, M.S., Kandil, E. and Matrougui, K. (2009). Protective Effects of Garlic and Silymarin on NDEA-Induced Rats Hepatotoxicity. *Int. J. Biol. Sci.,* **5**(6): 549-557.

[13] Snedecor, G.W. and Cochran, W.G. (1992). Statistical Methods, Sixth Edn. Oxford and IBH., New Delhi.

FIGURES

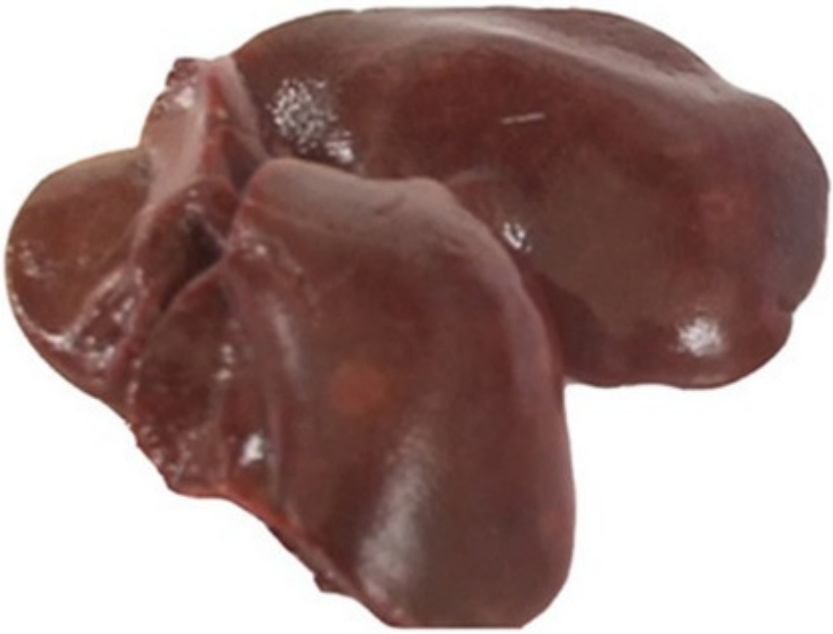

Fig 1. Liver from rat of group II showing diffused necrotic foci

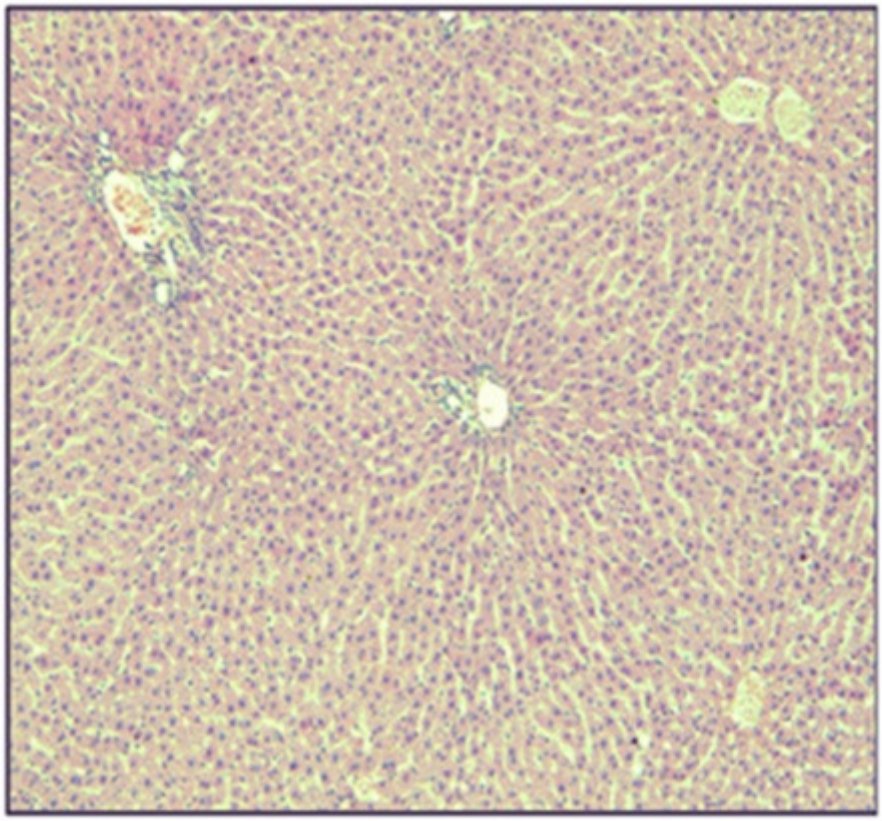

Fig 2. Section of liver from rat of group I showing normal architecture . H & E stain (x 150)

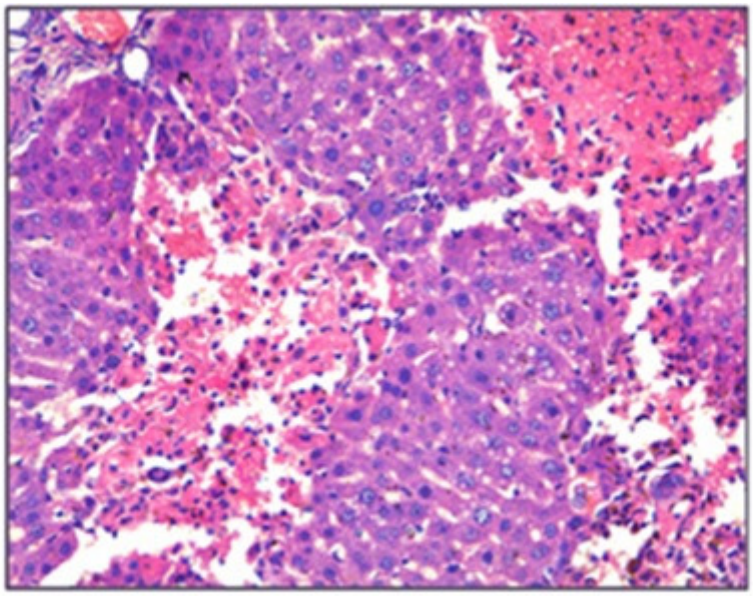

Fig 3. Section of liver from rat of group II showing necrosis and fatty changes. H & E stain (x 300)

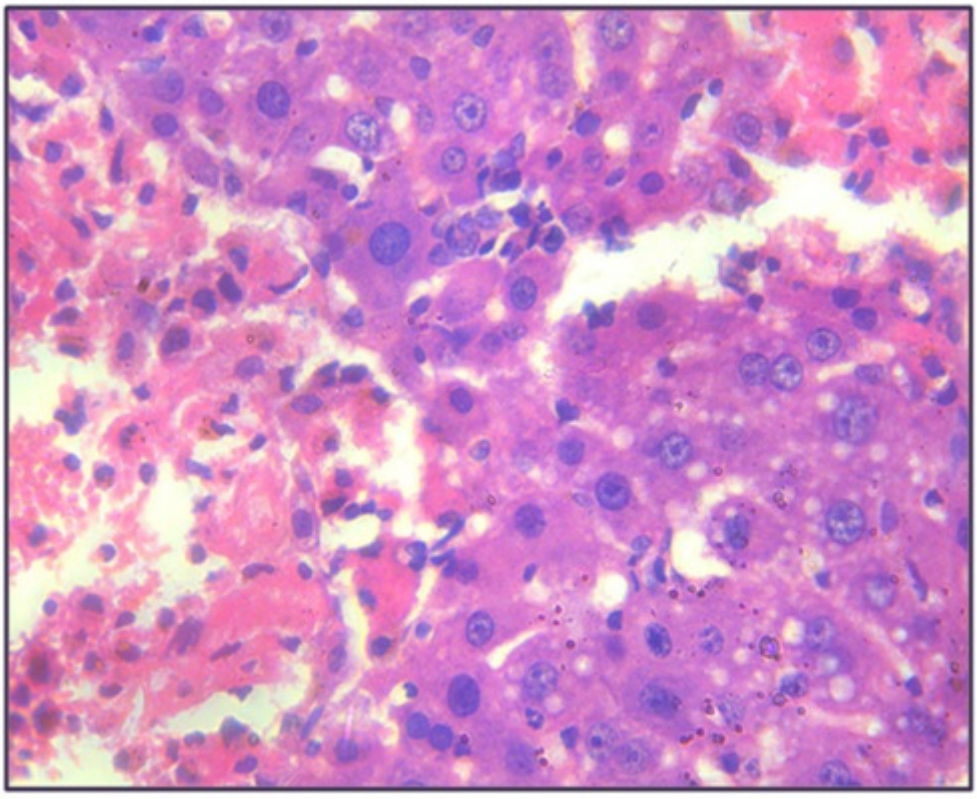

Fig 4. Section of liver from rat of group II showing cytomegaly and karyomegaly. H & E stain (x 600)

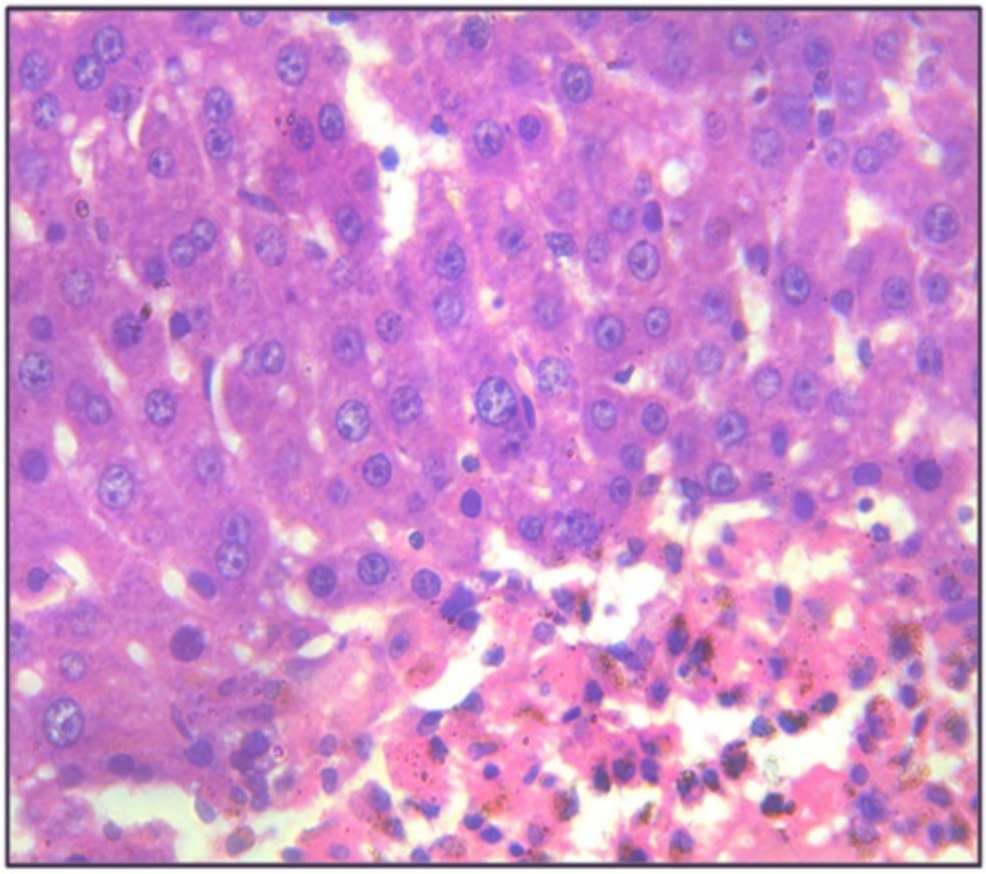

Fig 5. Section of liver from rat of group II showing more than one nucleolus. H&E stain (x 600)