

A preliminary study on serum liver function indices of Diethylnitrosamine induced hepatocarcinogenesis and chemoprotective potential of *Eclipta alba* in male Wistar rats

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Abstract

Aim: To study the effect of *E. alba* on liver function in experimental hepatocarcinogenesis in Wistar rats.

Materials and Methods: The study was designed to identify the protective effect of *Eclipta alba* in experimental diethylnitrosamine (DEN) induced hepatocarcinogenesis in rats. Sixty four male Wistar albino rats were randomly allotted to four groups of 16 rats each. DEN (0.01 %, v/v) was given in drinking water *ad libitum* and *E. alba* (50 mg/kg BW/day) was administered *per os* either alone or in combination for 120 days. Serum protein, albumin and liver functional status enzyme profiles were studied.

Results: Administration of DEN resulted in reduction in the body weights and DEN in combination with *E. alba* caused poor weight gain in male Wistar albino rats. Concurrent administration of *E. alba* with DEN significantly alleviated the effects of DEN on serum protein and albumin, while a partial protective effect was observed for other biochemical values and body weight.

Conclusion: The designed study could establish the partial protective effect of *E. alba* in experimentally induced hepatocarcinogenesis in rats.

Keywords: albumin, diethylnitrosamine, *E. alba*, hepatocarcinogenesis, liver functional status enzymes, serum protein, Wistar albino rats.

Introduction

With the advent of industrialization and modern technology, a large amount of synthetic compounds has been poured into man's biosphere as pharmaceutical products, cosmetics, food additives, agricultural chemicals etc. Most of these products gain entry into human or animal's body either deliberately or accidentally by ingestion. The liver is the first organ to be exposed to these chemicals. During this exposure, the absorbed chemicals undergo bio-transformation in the liver. In this process, liver may generate reactive or toxic metabolites and thus the liver is confronted with a higher concentration of potential toxins than any other organ in the body. Liver may respond to the bio-transformed toxic metabolites in different ways. Acute exposure to toxins results in breakdown of normal lipid metabolism, hepatocellular necrosis and/or hepatobiliary dysfunction, while prolonged chronic exposure usually results in cirrhosis or neoplastic transformations. Tumors of liver form one of the most common neoplasms encountered and hepatocellular carcinoma (HCC) accounts for 90% of all liver tumour incidences, ranking third in cancer related deaths [1]. The possibility of their formation in

gastrointestinal tract makes nitrosamines one of the interesting chemicals to be studied for the various effects in the mammalian body system. They are formed by the nitrosation of simple amine precursors in mammalian organism. The precursors of nitrosamines are almost ubiquitous in the environment, as they are contained in numerous foods, beverages, cosmetics, and medicines. The best studied nitrosamines like tobacco specific nitrosamines, dimethylnitrosamine, and diethylnitrosamine possess potential toxic and carcinogenic properties. Diethylnitrosamine (N-nitrosodiethylamine, DEN) is a well known hepatotoxin as well as hepatocarcinogen found in air, water and soil, and is normally used as a carcinogen to induce liver cancer in animal models [2, 3, 4]. Metabolic activation of DEN in liver produces ethylating electrophiles which react with DNA resulting in the formation of DNA adducts [5].

Recent advanced researches aiming to control malignancy by pharmacological intervention have reached new heights. Many synthetic and natural compounds have been discovered which can either arrest or reverse the process of malignancy significantly. *Eclipta alba* is an annual shrub which has been reported to be useful in liver ailments. It has been proved that *E. alba* possesses antioxidant as well as free radical scavenging properties. Moreover, it can

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Table- 1: Body weight of control and treated animals (mean \pm SE).

Groups	Initial body weights (g)	Final body weights (g)
Control	152.13 \pm 3.78	212.00 ^c \pm 8.34
DEN	152.13 \pm 3.19	92.00 ^a \pm 13.32
<i>E. alba</i>	152.00 \pm 3.16	216.33 ^c \pm 15.62
DEN+ <i>E.alba</i>	152.38 \pm 4.14	175.67 ^b \pm 4.33

P < 0.05, means with same superscript within a column do not differ from each other.

correct altered activity of Na⁺, K⁺ and ATPase associated with toxic injury [6].

Studies have confirmed its hepatoprotective activities against carbon tetrachloride and paracetamol induced hepatocyte damage in animals [7, 8, 9]. But a systematic research on any possible effect of *E. alba* on hepatocarcinogenesis seems to be scarce or absent [10]. In view of the above facts, this study investigated the protective effect of *E. alba* in experimentally DEN induced hepatocarcinogenesis in rats.

Materials and Methods

Ethical approval: The present study was conducted at the Department of Veterinary Pathology, Madras Veterinary College, Chennai. This study was approved by the Institutional Animal Ethics Committee.

Animals: Male albino rats (Wistar strain) of 7-9 weeks age weighing around 120-150 g obtained from Department of Laboratory Animal Medicine, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS) Madhavaram Milk Colony, Chennai, were used for the study. The animals were housed in polypropylene cages at a population density of six per cage. Animals were acclimatized for two weeks at Centralized Laboratory Animal House, Madras Veterinary College, with normal light and dark cycle. A commercial rat pellet diet (M/s Vet Care Ltd., Bangalore, India) and water were provided *ad libitum*.

Experimental protocol: Sixty four male albino rats of Wistar strain were weighed and randomly distributed into four groups, each group comprising of 16 rats. Group I consisted of normal control rats, maintained on commercial rat pellet diet and water; Group II, received 0.01% DEN (v/v) (Isopac®, 1g, Sigma Chemical Co. USA) in drinking water *ad libitum* for 120 days to induce hepatocarcinogenesis; Group III, treated with *E. alba* (50 mg/kg BW/day p.o (*per os*)) (Indian Herbs Research & Supply Co. Ltd, Saharanpur, India) in distilled water; Group IV, treated with 0.01% DEN (v/v) in drinking water *ad libitum* and *Eclipta alba* (50 mg/kg BW/day p.o) in distilled water simultaneously for 120 days. Animals were sacrificed at the end of experiment. The blood samples were collected for biochemical analysis and were allowed to clot and centrifuged at 1500 rpm for 20 min to separate the sera. Serum total protein and albumin were estimated by modified Biuret and Dumas method, aspartate

Table-2: Effect of *E.alba* on serum total protein, albumin and globulin of control and treated animals (mean \pm SE).

Groups	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
Control	6.70 ^b \pm 0.23	2.46 ^b \pm 0.03	4.06 \pm 0.27
DEN	5.40 ^a \pm 0.27	1.75 ^a \pm 0.14	3.65 \pm 0.31
<i>E.alba</i>	6.83 ^b \pm 0.23	2.51 ^b \pm 0.08	4.32 \pm 0.25
DEN+ <i>E.alba</i>	6.36 ^b \pm 0.21	2.43 ^b \pm 0.10	3.94 \pm 0.25

P < 0.05, means with same superscript within a column do not differ from each other.

aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) by IFCC (International Federation of Clinical Chemistry) method, gamma-glutamyl transferase (GGT) by Szasz method.

Histopathological study: Representative liver samples from each group were collected in 10% neutral buffered formalin. These samples were processed and stained by routine H&E stain for histopathological examinations following standard protocol.

Statistical analysis: The data obtained from different parameters were subjected to one-way analysis of variance using SPSS (Version 10.0 for windows) statistical software.

Results

The mean (\pm SE) values of body weights of adult male rats treated with DEN, DEN+*E.alba* and *E.alba* are presented in Table-1. Comparison of mean body weights revealed that there was no significant difference between the control and the *E. alba* group. In DEN group animals, there was a significant decrease in the final body weight when compared with control, *E. alba* and DEN+*E.alba*. The DEN+*E.alba* animals showed a significant increase in the final body weight when compared with DEN treated animals and a significant decrease when compared with control and *E. alba* groups.

The mean (\pm SE) values of total protein, albumin and globulin of adult male rats treated with DEN, DEN+*E. alba* and *E. alba* are given in Table-2. There was a significant decrease in the serum total protein and albumin in the DEN group when compared to the other groups. No significant difference was observed among the control, DEN, *E. alba*, DEN+*E.alba* groups for serum globulin. Table-3 shows mean (\pm SE) serum ALT, AST, ALP and GGT values in adult male rats of control, DEN, *E. alba* and DEN+*E.alba* groups. There was a significant increase in the levels of ALT, ALP and GGT in the DEN group when compared to the other groups. Significant decrease in the ALT, ALP and GGT values were observed in the DEN+*E.alba* group when compared with the DEN alone group. But there was a significant increase in the levels of serum ALT, AST and GGT in the DEN+*E. alba* treated groups when compared with control and *E. alba* alone groups.

Histopathological examinations revealed normal hepatic architecture of liver sections from control and *E. alba* alone group (Figure-1). Sections of liver from

Table-3: Effect of *E. alba* on serum ALT, AST, ALP and GGT of control and treated animals (mean±SE).

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)
Control	64.50 ^a ± 2.89	213.79 ± 7.31	841.25 ^a ± 20.16	2.58 ^a ± 0.24
DEN	142.60 ^c ± 11.62	250.47 ± 7.61	1502.59 ^b ± 181.34	31.69 ^c ± 4.32
<i>E. alba</i>	64.17 ^a ± 4.86	203.75 ^a ± 9.41	871.84 ^a ± 21.81	2.72 ^a ± 0.33
DEN+ <i>E. alba</i>	105.81 ^b ± 14.13	266.45 ^b ± 6.07	1053.66 ^a ± 104.41	11.69 ^b ± 4.37

P < 0.05, means with same superscript within a column do not differ from each other.

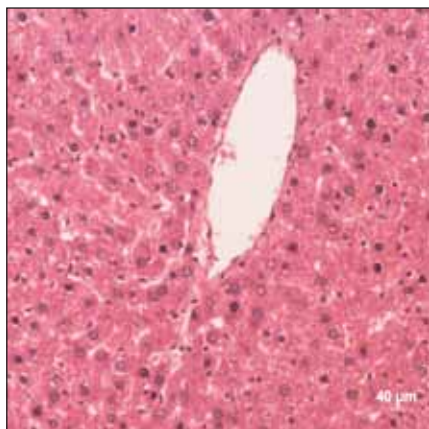


Figure-1: Liver-normal architecture, H & E Bar = 40µ.

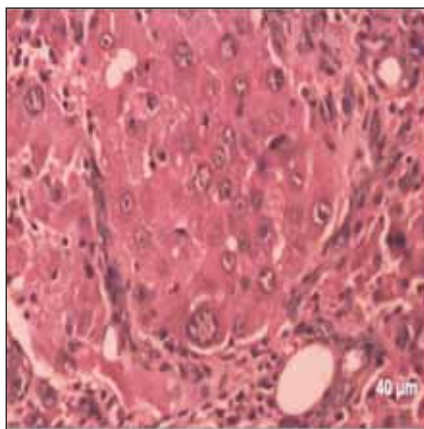


Figure-2: Neoplastic nodule with pleomorphic hepatocytes, atypical mitosis and atrophy of lining hepatocytes, H&E Bar = 40µ.

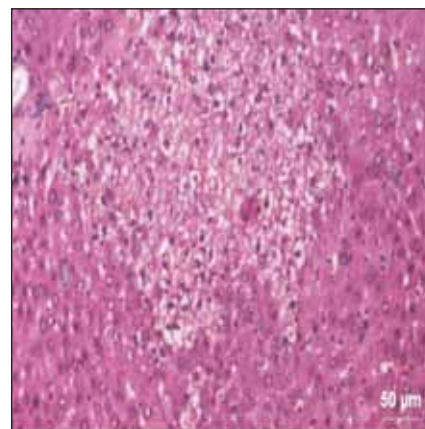


Figure-3: Clear cell foci in liver, H&E Bar = 50µ.

DEN treated group consistently showed characteristic hepatocellular carcinoma in all animals, with loss of normal architecture, pleomorphic hepatocytes forming neoplastic nodule, atypical mitosis and pressure atrophy of adjacent peripheral hepatocytes (Figure-2). Microscopical examinations of liver sections from DEN + *E. alba* showed a variation in histopathological picture. It varied from formation of clear cell foci (Figure-3), which was an early change in hepatocarcinogenesis (in 70% of animals) to the presence of hepatocellular carcinoma (in the rest 30% of animals).

Discussion

Treatment with DEN caused significant reduction in body weight of adult male rats, while poor weight gain was observed in the DEN+*E. alba* treated rats. However, the treatment with *E. alba* improved the weight gain by 39% when compared to the DEN alone treated rats. This clearly indicated that *E. alba* had a protective role over the DEN induced reduction in body weight gain of rats. Earlier studies [11, 12] also showed a significant reduction in body weight of rats treated with DEN after 11 weeks. The loss of weight induced by DEN is plausible because this compound specifically damages the liver which may result in the derangement of energy metabolism. The protective effect of *E. alba* on DEN induced reduction in body weights noticed in the present study could be attributed to the free radical scavenging activity of the *E. alba*, thus protecting the hepatocytes from free radical induced injury [6].

Reduction in serum total protein and albumin in DEN treated group could be ascribed to the develop-

ment of hepatic lesions observed in the present study that affected protein synthesis, which concurred with earlier findings [13]. The *E. alba* treatment protected the liver from the effects of DEN on serum protein and albumin. Significant increase in the ALT, ALP and GGT values were observed in the DEN treated group, when compared to the other groups. These findings are in accordance with the earlier reports of significant increase in ALT, AST and GGT values in rats fed with 0.01 percent DEN in drinking water *ad libitum* for 15 weeks [12]. Similar observations were made for ALT, ALP and GGT in rats dosed 10 mg DEN/ kg BW, five times a week for 15 weeks intragastrically [14]. The increase in the activities of serum ALT, ALP and AST occurred when hepatocellular damage caused abnormalities of liver function, and serum concentrations of these enzymes increased remarkably in hepatoma [12]. There was a 15 fold increase in the serum GGT values in the DEN treated rats when compared to the control rats, which was more than double fold increase in the serum GGT values recorded in previous studies. The GGT level increased with the emergence of neoplastic nodules and retained during differentiation of hepatoma. The elevated GGT level enabled the cells to respond to the proliferation by other stimuli and might facilitate nodule formation and tumour progression [15]. The increased activities of serum ALT, ALP, GGT and AST in the present study could be attributed to the hepatic lesions produced by DEN. Serum GGT levels of animals in DEN+*E. alba* group showed only five fold increase, which was well below the 15 fold increase encountered in DEN alone treated group. The significant increase in the serum ALT, GGT and AST values in the

DEN+*E.alba* group when compared to the control and *E. alba* alone treated rats and the significant decrease in the serum ALT and GGT values when compared to the DEN alone treated rats reflect the partial protective effect of *E. alba* on DEN induced hepatic lesion [16].

Conclusion

E. alba is one of the important ingredients of hepatoprotective ayurvedic formulations. The designed experiment elucidated the effect of this annual shrub on hepatocarcinogenesis. Eventhough the treatment with *E. alba* couldn't protect the liver completely, the present study revealed a partial protective effect of *E. alba* on DEN induced hepatocarcinogenesis as indicated by histopathological examinations and values of liver function indices. This can be attributed to the protective effect of *E. alba* on DEN induced oxidative cell membrane damage.

Authors' contributions

RA, SH, and CB designed the experiment. RA conducted the research experiment. RA, SH, and CB drafted and revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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