

the growth performance of *P. hysterophorus* through competitive and other indirect effects.

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## Protective effect of vitamin A, ascorbic acid and $\alpha$ -tocopherol on 2,4-dimethylaminoazobenzene-induced hepatoma in rats

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The chemoprotective effect of antioxidant vitamins (A, C and E) on chemically-induced hepatoma by 2,4-dimethylaminoazobenzene (DAB) in male albino rats has been studied. Group-I animals which were fed *ad libitum* with normal rat feed and water served as control. Each animal of the groups II–V received DAB injection intraperitoneally at a concentration of 20 mg/kg body wt once in a week. Immediately after administering the carcinogen, vitamins A (10,000 IU/kg body wt), C (250 mg/kg body wt) and E (400 mg/kg body wt) were given orally to groups III, IV and V, respectively and the experiment continued for a period of two months. Oral administration of antioxidant vitamins has a protective effect on the incidence of liver tumour monitored on the basis of liver weight, histological studies and enzymatic analysis. Administration of vitamins effectively protects the hyperplasia of the liver parenchymal cells and prevents damage of the nuclear envelope. Elevated levels of serum  $\alpha$ -glutamyl transpeptidase, acetylcholinesterase, GSH, ALP and bilirubin in the DAB-administered group were significantly reduced by administration of each vitamin.

DIET is the second major cause for cancer and induces cancer of colon, breast, stomach, liver, etc. Certain foods like high-fiber low-fat diets and fresh fruits and vegetables provide protection against cancer<sup>1</sup>. Fresh fruits and vegetables are a rich source of antioxidant vitamins like A, C and E that prevent cellular damage associated with cancer incidence. The scavenging capacity of the antioxidant vitamins prevents oxidative damage by neutraliz-

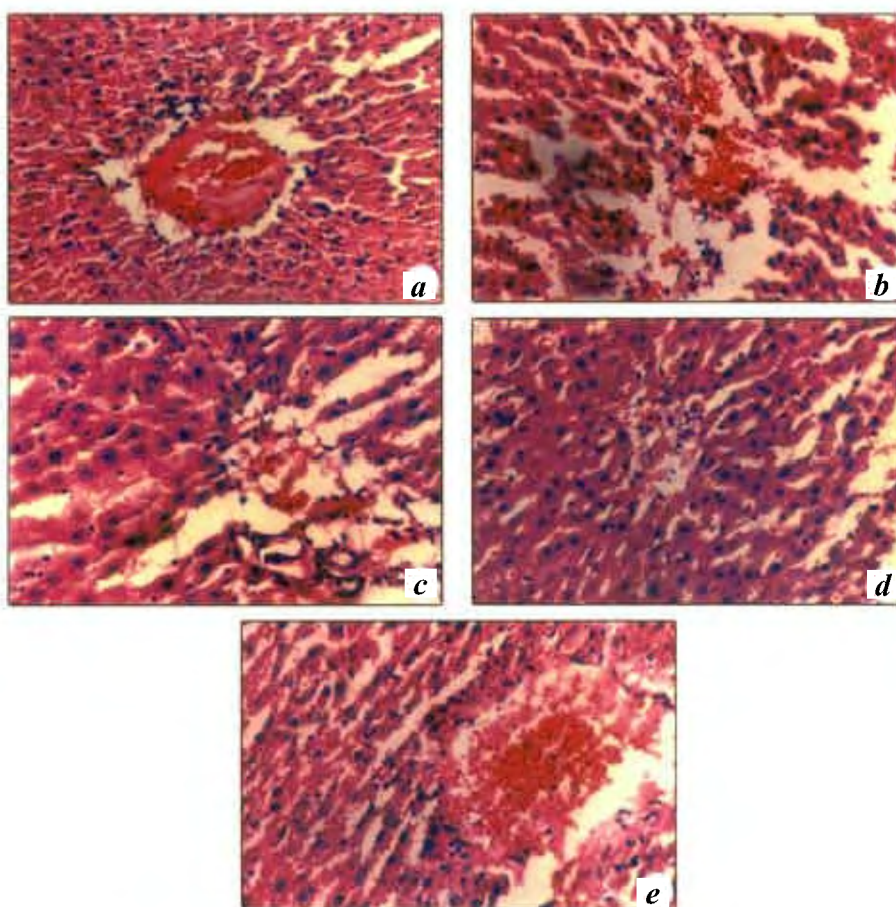
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ing the free radicals. Environmental carcinogens cause 50 to 90% of all cancers. The remaining 10% of cancers are caused by several other factors, not all of which are understood<sup>2</sup>. 2,4-dimethylaminoazobenzene (DAB) is commonly used as a colouring agent in foodstuff and is known to induce liver cancer (hepatoma) in experimental animals<sup>3,4</sup>. The present study was conducted to find out the protective effect of the antioxidant vitamins A, C and E on hepatoma induced by DAB.

Male albino rats ( $n = 25$ ) of Wistar strain ( $170 \pm 20$  g) were acclimatized for 15 days and fed *ad libitum* with rat feed and water. These animals were divided into five groups. Group I served as control. Carcinogenesis was induced in the groups II–V by administering intraperitoneally 20 mg/kg body wt of DAB once in a week for a period of two months. Soon after DAB administration, vitamins A (10,000 IU/kg body wt), C (250 mg/kg body wt) and E (400 mg/kg body wt) were orally given to groups III, IV and V, respectively. During the ninth week, animals were anaesthetized by ether, the blood was collected by cervical decapitation and the serum was separated for assay of the enzymes. Livers were excised and weighed and then homogenized with phosphate

buffer. The homogenates were centrifuged at 10,000 g for 30 min. The supernatant was taken for biochemical analysis. Liver-tissue sections were fixed in 10% formalin and were stained with haematoxylin and eosin for histological studies by light microscopy. Assay of  $\alpha$ -glutamyl transpeptidase<sup>5</sup>, acetylcholinesterase<sup>6</sup>, glutathione<sup>7</sup>, alkaline phosphatase<sup>8</sup>, glutathione-s-transferase (GST)<sup>9</sup>, bilirubin<sup>10</sup> and protein<sup>11</sup> was done using standard methods. Statistical analysis of the data was performed with Students 't' test.

Hyperplasia of hepatic parenchymal cells in DAB-administered rats has been observed. Normal rats show more compact and well-distributed junctional complexes (Figure 1 *a*). In DAB-administered rats, hepatic cells exhibit loss of contact inhibition (polarity) and damaged central vein of liver lobules (Figure 1 *b*). However, animals treated with vitamins (Figure 1 *c–e*) show higher cell density with compact junctional complexes than DAB-administered animals. Hepatic cells of the normal rats have well-differentiated cytoplasm with polymorphism of nuclei, but in DAB-administered rats, the nuclear envelope is damaged. However, the protective effect of treatment with vitamins has been shown in



**Figure 1.** Histological appearance of liver of rat. *a*, Normal; *b*, DAB alone; *c*, DAB + vitamin A; *d*, DAB + vitamin C; *e*, DAB + vitamin E. Sections stained with haematoxylin and eosin.

**Table 1.** Effect of antioxidant vitamins on liver weight,  $\alpha$ -glutamyl transpeptidase activity, acetylcholinesterase activity and GSH level of rats administered with DAB

Group	Animal status	Liver wt/100 g body wt	$\alpha$ -glutamyl transpeptidase activity (IU/l)	Acetylcholinesterase activity (IU/l)	GSH (nmol/mg protein)
I	Normal rats	2.44 $\pm$ 0.27	1.18 $\pm$ 0.2	50.1 $\pm$ 3.59	10.92 $\pm$ 0.11
II	DAB alone	6.3 $\pm$ 0.70	21.1 $\pm$ 2.1	579.4 $\pm$ 31.66	28.1 $\pm$ 3.20
III	DAB + vitamin A	3.58 $\pm$ 0.15*	7.5 $\pm$ 0.35*	312.0 $\pm$ 21.35*	16.5 $\pm$ 2.5*
IV	DAB + vitamin C	3.14 $\pm$ 0.32*	2.1 $\pm$ 0.5*	72 $\pm$ 10.5*	12.3 $\pm$ 2.22*
V	DAB + vitamin E	3.72 $\pm$ 0.47*	8.1 $\pm$ 0.9*	200 $\pm$ 30.5*	15.5 $\pm$ 2.5*

\* $P$  < 0.001 vs group II; Values are mean  $\pm$  SD;  $n$  = 5.

**Table 2.** Effect of antioxidant vitamins on ALP, GST and bilirubin levels of rats administered with DAB

Group	Animal status	ALP (KAU/dl)	GST (nmol/min/mg protein)	Bilirubin (mg/dl)
I	Normal rats	23.6 $\pm$ 2.3	728 $\pm$ 15	0.52 $\pm$ 0.05
II	DAB alone	43.6 $\pm$ 2.7	1211 $\pm$ 100	1.85 $\pm$ 0.40
III	DAB + vitamin A	30.9 $\pm$ 2.5*	860 $\pm$ 80*	0.70 $\pm$ 0.08*
IV	DAB + vitamin C	25.7 $\pm$ 2.5*	750 $\pm$ 50*	0.65 $\pm$ 0.10*
V	DAB + vitamin E	28.7 $\pm$ 3.6*	1020 $\pm$ 70*	1.0 $\pm$ 0.27*

\* $P$  < 0.001 vs group II; Values are mean  $\pm$  SD;  $n$  = 5.

reducing the cancer of the liver. Liver weight of DAB-administered rats increased when compared to normal rats. Vitamin administration significantly lowered the liver weight by reducing the hyperplasia of liver cells (Table 1). Increased activity of  $\alpha$ -glutamyl transpeptidase, a marker enzyme of hepatoma<sup>12</sup> in the serum was found to be effectively lowered by vitamin treatment. Acetylcholinesterase, GSH, ALP, GST and bilirubin levels, which increased after DAB administration, were found to be lowered by vitamins (Tables 1 and 2).

Free radicals are believed to play a part in tumour development and growth<sup>13</sup>. Hence interest in the usefulness of antioxidant nutrients in the treatment of cancer has increased. The antioxidant vitamins A, C and E may also have biological activities other than free-radical trapping, that relate to their cancer-preventive properties. These biological-activity mechanisms include immune stimulation, inhibition of nitrosamine formation, enhancement of cell communication and influence on metabolic activation of carcinogen<sup>14</sup>. Vitamin A upregulated the expression of connexin 43 gene (pore-like structures) responsible for the production of one of the most important components of the gap junction<sup>15</sup>. High doses of vitamin C are effective in selectively killing tumour cells<sup>16</sup> and vitamin C has been suggested to have a potential anticancer effect<sup>17</sup>. High supplement levels of vitamin E inhibit ethanol-induced free-radical activity, formation of free-radical products and suppress the promotion of cancer by ethanol<sup>18</sup>. Vitamin E supplementation also reduces the risk of chemical and radiation-induced cancers<sup>19</sup>. In conclusion, high doses of antioxidant vitamins (A, C and E) were found to be protective against DAB-induced liver cancer.

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