



Preventive effect of *Sphaeranthus indicus* Linn. on *p*-Dimethylaminoazobenzene Induced Hepatocarcinogenesis in Male Albino Rats

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ABSTRACT

The present study aims to evaluate preventive effect of *S.indicus* on *p*-dimethylaminoazobenzene (DAB) induced hepatocarcinogenesis in rats. Oral administration of stem ethanol extract of the *S.indicus* (300mg/kg) effectively prevent the hepatocarcinogenesis as exposed by reduce in the levels of extend of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphate (ALP), total bilirubin, total cholesterol, lipid peroxidase (LPO) and raise the level of glutathione-S-transferase, superoxide dismutase (SOD) and catalase (CAT) when compared to DAB induced rats. The histopathological changes of the liver sample compared with respective control. The present study shows a significant preventive effect of *S.indicus* (SI) on DAB administrated hepatocarcinogenesis in rats.

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Introduction

Hepatocellular carcinoma is the most common liver cancer in the world. The world incidences are 14.7 and 4.92/100,000 population in males and females¹. The incidence of hepatocellular carcinoma in developed countries, 7.64 and 2.65/100,000 population in male and female, is lower than that of developing countries, 17.84 and 6.17/100,000 population in male and female². Diet is the second major cause for cancer and induces cancer of colon, breast, stomach, liver, etc³. Environmental carcinogens cause 50 to 90% of all cancers. The remaining 10% of cancer caused by several other factors, not all of which are understood⁴. *p*-Dimethylaminoazobenzene is commonly used as a colouring agent in foodstuff and is known to induce liver cancer in experimental animals⁵. Phenobarbital is an anti-epileptic drug and is also of carcinogenic potential⁶ to human, mice and rats when administered orally^{7,8}. It also strong a strong induced of *p*-450 enzymes like CYP2B1 and CYP2B2 which in turn enhance hepatic tumors. The chronic feeding of both *p*-DAB and PB has been reported to promote tumor growth⁹. Many researchers are concluded from their research that in recent years complementary and alternative medicines have been gaining ground as a supportive medicine in cancer therapy.

Sphaeranthus indicus Linn. (Asteraceae) popularly known as 'Gorakmundi' is cultivated all over India¹⁰ for its medicinal values. It traditionally used to treat the diseases like, allergy, inflammation, diabetes, and oxidative stress^{11,12}. Our detailed review shows no information on the preventive effect of *S.indicus* methanolic extract of stem in hepatocarcinogenesis induced by DAB in albino rats, so the present investigation deals with its protective efficiency of hepatocarcinogenesis induced by DAB in albino rats.

Materials and methods

Chemicals-4-Dimethyl amino azo benzene, Bovine Serum albumin, Bilirubin, 1', 3,3'-tetramethoxy propane, Sodium

pyruvate, Epinephrine, 1-chloro-2, 4-dinitrobenzene, Reduced glutathione, Thiobarbituric acid (TBA) were obtained from Sigma Chemical Company, ST. Louis, MO, USA. All other chemicals and reagents (analytical grade) used in this present study were purchased from Glaxo Laboratories, Mumbai, SD Fine Chemicals, Mumbai and Sisco Research Laboratories, Pvt. Ltd., India .

Animals-Albino rats (Male) of Wistar strain (150±10gm), procured and they were kept in polypropylene cages bedded with husk and were acclimatized in the animal house of the Department of Biochemistry (under the supervision of Institutional Animal Ethics Committee), J.J. College of Arts and Science, Pudukkottai.

Plant extract-The stem of *S.indicus* collected from the Medicinal garden of J. J. College of Arts and Science, Pudukkottai. The ethanolic extract was prepared by suspending the dried powder of stem in ethanol for 48 hours and filtered. Then the residue was again dissolved with ethanol and filtered. Both filtrate combined and concentrated. The extract was dissolved in saline (0.8%) and used for the *in vivo* experiments.

Experimental design

The rats were divided into three groups with 6 rats as follows:

Group I: Control

Group II: DAB induced rats (DAB 0.06% for 30 days by mixing with fed and then 0.05% Phenobarbital for 30 days by mixing with water).

Group III: DAB + SI ethanol extract 150mg/Kg b.wt. for 60 days orally.

Group IV: DAB + SI ethanol extract 300mg/Kg b.wt. for 60 days orally.

At the end of the experiment all rats were sacrificed by cervical decapitation and the serum was collected from the collected blood. Immediately liver was removed and washed with ice-cold physiological saline. They were homogenized in

0.1M tris-HCl buffer pH 7.4 to give a 10% homogenate. This homogenate used for the appropriate parameter estimation.

The biochemical parameters, (Bilirubin¹⁴, Cholesterol¹⁵ & Protein content¹⁶), marker enzymes (Alanine aminotransferase¹⁷, Aspartate aminotransferase¹⁷, Alkaline phosphatase¹⁸) and antioxidant parameters (Lipid peroxidation¹⁹, Catalase²⁰, Superoxide dismutase²¹ and Glutathione-S-transferase²²) were estimated in serum. Small portion of the liver was removed and fixed in 10% formalin for the histopathological study²³.

Statistical analysis of data

Values are mean \pm SD for six animals in each group. Statistically significant changes in different groups were evaluated by student's 't' test. The levels of significance were denoted by 'p' values.

Results

The DAB administrated rats had hepatocarcinogenesis and the SI treated rats were recovered from the hepatocarcinogenesis, which was compared from Group I rats (Fig.1). The level of total protein in serum of Group II DAB induced rats showed a significant ($p < 0.05$) increase when compared with Group I control rats. On the other hand group III and IV rats, which received oral administration of SI extract showed a significant ($p < 0.05$) decrease in the level of total protein in serum sample when compared with group II DAB induced rats (Table 1).

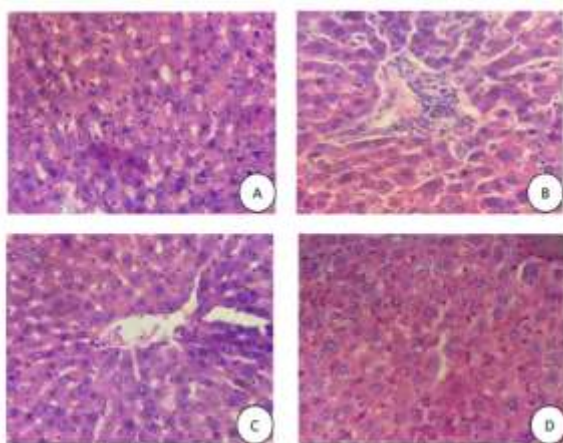


Figure 1. A-Normal; B-DAB Alone; C-DAB+SI (150mg/Kg); D-DAB+SI (300mg/kg), Sections stained with Haematoxylin & Eosin.

Histopathological studies of liver tissue shows following features,

- The H&E stained liver tissue section shows normal cells.
- The liver tissue cells are round to oval with hyper chromatic nucleus, the portal triad shows increased chronic inflammatory cells, inter cellular area shows edematous changes.
- This section shows reduced inflammatory cells in the portal triad, inter cellular edema also reduced, the cells are apparently appears normal.
- The liver tissue section shows mild cellular changes, scanty inflammatory cells.

Table 1 depicts the activity of the total bilirubin in serum sample of control and experimental rats. The level of total bilirubin in serum of group II DAB induced rats showed a significant ($p < 0.05$) increase when compared with group I control rats. On the other hand group III and IV rats, which received oral administration of SI extract showed a significant ($p < 0.05$) decrease in the level of total bilirubin in serum sample when compared with group II DAB induced rats. The level of total cholesterol in the serum of control and experimental rats is

given in table 1. In DAB group II rats, a significant ($p < 0.05$) increase in the content of total cholesterol in serum was observed when compared with Group I rats maintained as control. Oral administration of the extract of SI to the group III and IV rats showed a significant decrease in the level of total cholesterol serum, when compared with group II DAB induced rats.

Table 2 shows the level of SGOT in serum of control and experimental rats, the level of SGOT in serum sample of group II DAB induced rats showed a significant ($p < 0.05$) increase when compared with group I control rats. On the other hand group III and IV rats, which received the oral administration of SI extract showed a significant ($p < 0.05$) decrease in the level of SGOT in serum, when compared with Group II DAB rats. Results tabulated in table 2 showed a significant ($p < 0.05$) increase in the level of SGPT in serum of DAB induced Group II rats, when compared with Group I control rats. On comparison the Group III and Group IV rats with the group II DAB induced rats, a significant ($p < 0.05$) decrease in the level of SGPT were observed.

Table 2 depicts the activity of the ALP in serum sample of control and experimental rats. The level of ALP in serum of Group II DAB induced rats showed a significant ($p < 0.05$) increase when compared with group I control rats. On the other hand group III and IV rats, which received oral administration of SI extract showed a significant ($p < 0.05$) decrease in the level of ALP in serum sample when compared with group II DAB induced rats. The level of Lipid peroxidation in the liver tissues of control and experimental rats is given in table 3. In DAB group II rats, a significant ($p < 0.05$) increase in the content of lipid peroxidation in liver tissues was observed when compared with group I control rats.

Oral administration of the extract of SI to the group III and IV showed a significant ($p < 0.05$) decrease in the level of lipid peroxidation of liver, when compared with group I DAB induced rats. Table 3 shows the levels of GST in liver tissues of control and experimental rats. The level of GST in liver tissues of group II DAB induced rats showed a significant ($p < 0.05$) decrease, when compared with group I control rats. On the other hand group III and IV rats, which received the oral administration of SI extract showed a significant ($p < 0.05$) increase in the level of GST in liver, when compared with group II DAB induced rats.

Table 4 showed a significant ($p < 0.05$) decrease in the level of SOD in liver of DAB induced group II rats, when compared with group I control rats. On comparison the group III and IV rats with the group II DAB induced rats, a significant ($p < 0.05$) increases in the level of SOD were observed. Results of table 4 shows the level of catalase in liver tissues of control and experimental rats, the level of catalase in liver tissue of group II DAB induced rats showed a significant ($p < 0.05$) decrease, when compared with group I control rats. On the other hand group III and IV and group IV rats, which received oral administration of SI extract showed significant ($p < 0.05$) increase in the level of catalase in liver tissues when compared with group II DAB induced rats.

Discussion

Natural products can modify carcinogenesis in different ways, such as modification of phase-1 enzyme for carcinogen activation, detoxification of carcinogen through phase-2 enzymes, scavenging DNA agent, suppressing proliferation of early, preneoplastic lesions, or inhibition of certain properties of

cancer cell²⁴. No significant alteration in body weight, body weight gain or food consumption in normal rats. In contrast, body weight and food consumption were significantly reduced in DAB induced group of animals. Pretreatment of animals with ethanolic extract of SI may increase body weight. Hyperplasia of hepatic parenchyma cells in DAB administered rats has been observed in DAB administered animals, hepatic cell exhibit loss of contact inhibition (polarity) and damaged central vein of liver lobules and the nuclear envelop is damaged.

Acetylcholine esterase, GHS, ALP, GST and bilirubin levels are increased after administration p-DAB²⁵. Increase in serum total protein level is due to the DAB administration. In the present study SI extract has been found to increase the level of total protein in the treated group. Serum bilirubin levels are related to the status and function of hepatic²⁶. The elevated level of total bilirubin is indicative of poor hepatic function²⁷ DAB. The level was decreased in *Sphaeranthus indicus* treated group III and group IV rats in the present study may due to the hepatic damage induce the increase in total bilirubin in serum. Cholesterol metabolism is a normal regulation process, which first transfer deoxycholic acid through biliary system in liver, then as bile salt was passed in biliary system. When hepatic inadequacy on cancer and chronic liver disease, form esterification and evaluation of cholesterol, which causes changes of total cholesterol level²⁸. Total cholesterol level increased in DAB induced rats in-group II compared with Group I normal rats. Treatment with SI extract had effectively controlled the rate of total cholesterol in serum, which suggests that the effect of SI control total cholesterol.

Serum enzyme levels are not a direct measure of hepatic injury they show the status of liver. The elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. High level of SGOT indicates liver damage due to DAB administration. SGPT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore SGPT is more specific to the liver and a better parameter for detecting liver cancer²⁹ SI at the dose of 150 and 300mg/Kg decreased the level of both SGOT and SGPT significantly and is a dose dependent manner.

Serum ALT levels are also related to the status and function of hepatic cells, increase in serum ALP is due to increased synthesis, in presence of increasing biliary pressure. ALP level of DAB administered rats increased when compared to normal rats. SI administration significantly lowered the ALP level. In hepatocellular carcinoma there is disequilibrium between oxidant and anti-oxidant balance, which is tilted towards oxidant side. This oxidative stress may be the reason for the elevated LPO level in the liver. LPO may lead to the formation of several toxic by products such as 4-hydroxy nonenal and melanodialdehyde, which can attack cellular targets including DNA, inducing mutagenicity and carcinogenesis²⁷.

The level of lipid peroxides was significantly elevated by the administration of DAB, these elevated levels were lowered by the administration of SI extract. One of the major mechanisms of chemical protection against carcinogenesis, mutagenesis, and other forms of toxicity mediated by electrophiles is the induction of enzymes involved in their metabolism; particularly phase 2 enzymes such as glutathione-s-transferase (GSTs), uridine diphosphate, etc. Furthermore, induction of phase 2 enzymes appears to be a sufficient condition for obtaining chemoprevention and can be achieved in

many target tissues by administering any of a diverse array of naturally occurring and synthetic chemical agents³⁰. The GST level of DAB administered rats decreased when compared to normal rats. After the SI administration significantly increase the GST level in this study.

SOD is a mitochondrial enzyme, which is found to quench free radicals and prevents tissue damage, decrease in activity of SOD leads to decreased production of hydrogen peroxides³¹. In our study a significant decrease in SOD level has been observed in DAB treated animals, the present study indicates that the simultaneous administration of SI may increase the SOD level. The enzymatic antioxidant defense systems are natural protective barriers against lipid peroxidation, SOD and CAT are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent the generation of hydroxyl radicals and protect the cellular constituents from oxidative damage³².

In the present study, the administration of DAB altered the biochemical parameters and marker enzyme in the DAB treated rats. Administration of ethanol extract of SI along with the DAB induced rats showed a significant control of the hepatocarcinogenesis process in rats. Further studies are warranted to isolate the active principles from the *Sphaeranthus indicus*.

References

1. Bosch F X, Ribes J and Borrás J. Epidemiology of primary liver cancer, *Semin. Liver Dis.* 19 (1999) 271.
2. Adrian M and Bisceglie MD. Epidemiology and clinical presentation of hepatocellular carcinoma. *J Vasc Interv Radiol*, 13 (2002) S169.
3. Karol S. Cancer drugs development in the post genomic age. *Current science*, 81 (2001) 549.
4. Manu L, & Metha L A, *The Nature of Cancer*, Kothari Publications, Bombay, (1973) 23.
5. International Agency for Research on Cancer, *World Health Organization, Monographs*, 1-60 (1987) 7.
6. International Agency for Research on Cancer (IARC), *Summaries and Evaluations, Phenobarbital and its sodium salt*, Group 2B, 79 (2001) 161.
7. Kitagawa T, Pitot H C, Miller E C & Miller J A, Promotion by dietary Phenobarbital of hepatocarcinogenesis by 2-methyl-N, N-dimethyl-4-aminoazobenzene in the rat, *Cancer Res*, 39 (1979) 112.
8. Kitagawa T & Sugano H, Enhancement of azodye hepatocarcinogenesis with dietary pehnobarbital in rats, *Gann*, 68 (1977) 255.
9. Biswas S J, Pathak S & Khuda-Bukhash A R, Assessment of the genotoxic and cytotoxic potential of an anti-epileptic drug. Phenobarbital, in mice: A time course study, *Mutat Res*, 563 (2004) 1.
10. Warriar P M, Nambiar V P K & Raman Kutty C, In: *Indian Medicinal Plants*, Vol. I, (Orient Longman, Hyderabad, India) 2004, 180.
11. Yoganarasimhan S N, In. *Medicinal plants of India-Tamil Nadu*, Vol.2, (Cyber Media, Bangalore, India) 2000, 512.
12. Nadkarni A K, In. *Indian material medica*, Vol. I, (Popular Prakashan Ltd., Mumbai, India) 2000, 113.
13. Trease E G & Evans W C, *Textbook of Pharmacognosy*. 3rd ed. London, Baillere Tindal. 81-90 (1989) 268.
14. Malloy H T & Evelyn K A, The determination of bilirubin with the photoelectric colorimeter, *J. Biol. Chem.*, 119 (1937) 481.

15. Abell L L, Levy B B, Brodie B B & Kendall F E, A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity, *J. Biol. Chem.*, 195 (1952) 357.
16. Lowry O H, Rosebrough N J, Farr A L & Randall R J, Protein measurement with the Folin-phenol reagent, *J.Biol.chem*, 193 (1951) 265.
17. King J, *The transaminases: Alanine and aspartate transaminases*; In: Van.D.(ed.) Nostrand co., London pp. (1965) 121.
18. King J, *The hydrolases, acid and alkaline phosphatases. In: Practical Clinical Enzymology*, Van, D. (ed.), Nostarand Co., London, pp. (1965b) 191.
19. Okhawa H, Ohishi N & Yagi K, Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal. Biochem*, 95 (1979) 351.
20. Takahara S, Hamilton B H, Nell J V, Kobara T Y, Ogura Y & Nishimura E T, Hypocatalasemia: a new genetic carrier state. *J.Clin. Invest.*, 29 (1960) 610.
21. Misra H P & Fridovich I, The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase, *J. Biol. Chem.*, 247 (1972) 3170.
22. Habig W H, Pabst M J & Jacob W B, Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation, *J.Biol. Chem.*, Vol.249 (1974) 7130.
23. Galigher A E & Kayloff E N, *In: Essentials of practical microtechniques. Philadelphia: Lea and Febiger.* pp. (1971) 77.
24. Naoto U, Naoki K, Isao S & Eikai K, Aged Garlic extract inhibits development of putative preneoplastic Lesion in rat hepatocarcinogenesis, *The Journal of Nutrition.* (2006) 855S.
25. Antony Joseph Velangannin A & Balasundaram C, Protective effect of vitamine A, ascorbic acid and alpha-tocoperal on 2,4-dimethyl amino azobenzene-induced hepatoma in rats, *Current science*, 85 (2) (2003) 201.
26. Susanta K M, Goutam C, Malaya G & Upal K M, Hepatoprotective activity of *Diospyros malabarica* bark in carbon tetra chloride intoxicated rats. *European Bulletin of Drag Research.*13 (1) (2005) 25.
27. Balasubramanian R, Narayanan M, Dechen C & Dhanapal S, Chemoprevention of N-Nitrosodiethylamine induced Phenobarbital promoted Liver tumors in rat by extract of *Indigofera aspalathiodes*, *Biol.Pharm.Bull.*28 (2) (2005) 364.
28. Jung T J, Niang X, Xiao Y Z & Chang P, Lipids Changes In Liver Cancer, *J Zhejiang Univ Sci B*, 8 (6) (2007) 398.
29. Susanta K M, Goutam C, Malaya G & Upal K M, Hepatoprotective activity of *Diospyros Malabarica* bark in carbon tetrachloride intoxicated rats, *European bulletin of drug research*, 13 (2005) 25.
30. Thomas W K, Chemoprevention By Inducers Of Carcinogen Dissociation Enzymes, *Environmental Health Perspectives*, 105 (1997) 965.
31. Attri S, Rana S V, Vaiphie K, Katyal R, Sodhi C P, Kanwar S, & Singh, Protective Effect Of N- Acetylcysteine In Isoniazid Induced Hepatic Injury In Growing Rats, *Indian.J. Esp Boil*, 39 (2001) 436.
32. Balamurugan M, Parthasarathi K, Cooper E L & Ranganathan L S, Earthworm paste (*Lampito mauritii*, Kinberg) alters inflammatory, oxidative, haematological and serum biochemical indices of inflamed rat, *European Review for Medical and Pharmacological Sciences*, 11(1) (2007) 77.

Table-1 Activity of Bodyweight, Protein, Bilirubin & cholesterol in serum of control and experimental group of rats

S.No	Parameters	Group I Normal	Group II DAB induced	Group III DAB induced + SI Extract (150mg/Kg b.w.)	Group IV DAB induced + SI Extract (300mg/Kg b.w.)
1.	Body Weight	126±4.32	92 ± 2.18 ^{a*}	103±5.10 ^{a*bNS}	109 ± 1.29 ^{b**}
2.	Total Protein	158±6.41	98 ± 3.11 ^{a*}	105±2.15 ^{a*bNS}	145±3.60 ^{b***}
3.	Total Bilirubin	2.46±0.14	15.66 ± 0.54 ^{a*}	11.68±0.36 ^{a*bNS}	5.7±1.38 ^{b**}
4.	Total Cholesterol	0.23±0.06	2.08 ± 0.34 ^{a*}	1.69 ±0.024 ^{a*b*}	1.13 ±0.05 ^{b**}

a-Group II, III and IV compared with Group I; b-Group III and IV compared with Group II (P<0.05, NS-Not Significant)

Table-2 Activity of Marker Enzymes in serums of control and experimental group of rats

S.No	Parameters	Group I Normal	Group II DAB induced	Group III DAB induced + SI Extract (150mg/Kg b.w.)	Group IV DAB induced + SI Extract (300mg/Kg b.w.)
1.	SGOT	116±1.15	251±6.00 ^{a*}	194 ± 7.40 ^{a*b*}	141 ± 3.74 ^{b**}
2.	SGPT	36±2.16	59±2.64 ^{a*}	44 ± 1.29 ^{a*b*}	38.20 ± 1.24 ^{b***}
3.	ALP	1.5±0.06	4.6±0.47 ^{a*}	2.6 ± 0.47 ^{a*bNS}	1.8 ± 0.87 ^{b*}

a-Group II, III and IV compared with Group I; b-Group III and IV compared with Group II (p<0.05, NS-Not Significant)

Table-3 Activity of Lipid peroxidation and glutathione-S-transferase in Liver Tissues of control and experimental group of rats

S. No	Parameters	Group I Normal	Group II DAB induced	Group III DAB induced + SI Extract (150mg/Kg b.w.)	Group IV DAB induced + SI Extract (300mg/Kg b.w.)
1.	Lipid Peroxidation	1.503±0.312	4.013±0.782 ^{a*}	3.213±0.125 ^{a* b*}	2.012±0.331 ^{b*}
2.	Glutathione-S-transferase	0.25±0.50	0.09±0.02 ^{a*}	0.14±0.20 ^{a* bNS}	0.22±0.32 ^{b*}

a-Group II, III and IV compared with Group I; b-Group III and IV compared with Group II (p<0.05, NS-Not Significant)

Table-4 Activity of Antioxidants in Liver Tissues of control and experimental group of rats

S. No	Parameters	Group I Normal	Group II DAB induced	Group III DAB induced + SI Extract (150mg/Kg b.w.)	Group IV DAB induced + SI Extract (300mg/Kg b.w.)
1.	SOD	6.14±0.23	1.97±1.07 ^{a*}	3.92 ± 0.57 ^{a* bNS}	5.02 ± 0.51 ^{b*}
2.	CAT	67.35±2.13	44.2±1.93 ^{a*}	52.51 ± 0.36 ^{a* b*}	60.61 ± 0.53 ^{b*}

a-Group II, III and IV compared with Group I b-Group III and IV compared with Group II (p<0.05, NS-Not Significant)