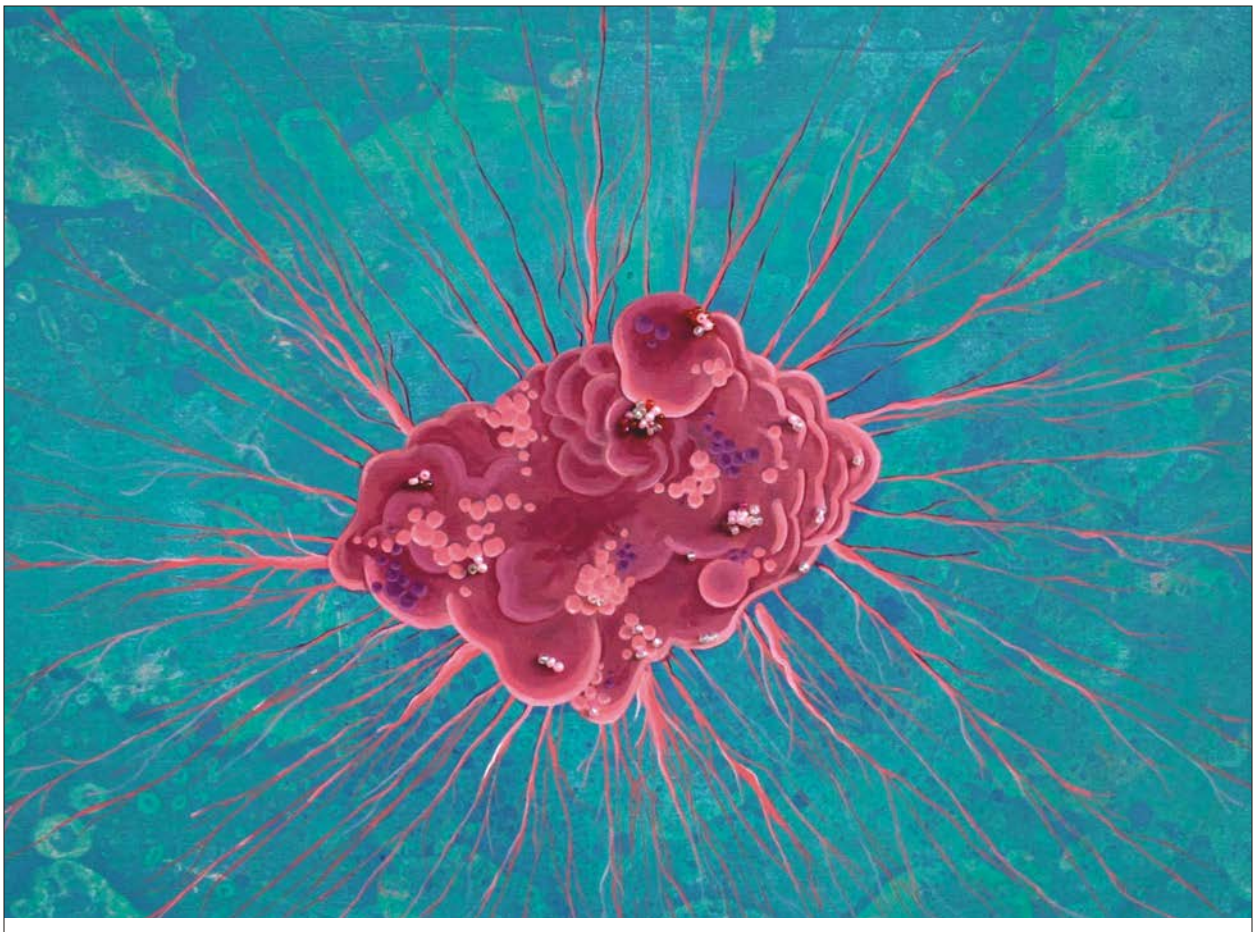

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DEN-Induced Cancer and its Alleviation by *Anisomeles malabarica* (L.) R.Br. Ethanolic Leaf Extract in Male Albino Mice

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Abstract: *Anisomeles malabarica* (L.) R. Br. is a traditional medicinal plant of the Labiatae family, distributed throughout India. It has been used in folk medicine for the treatment of cancer and liver disorders. The aim of this work is to study the anticancer effect of crude ethanolic leaf extract of *Anisomeles malabarica*. Its anticancer activity was evaluated on Diethylnitrosamine (DEN) in mice. The assessment of anticancer activity was evaluated by measuring the activities of total protein, Glutamate Pyruvate Transaminase (GPT), Glutamate Oxaloacetate Transaminase (GOT), Acid Phosphatase (ACP) and Alkaline Phosphatase (ALP). The ethanolic extract at an oral dose of 100 mg kg^{-1} exhibited a significant ($p<0.05$) protective effect by reduce liver and serum levels of total protein, GPT, GOT, ACP and ALP as compared to DEN induced mice. These biochemical observations were supplemented by histopathological examination of liver sections. Thus it could be concluded that ethanolic extract of *Anisomeles malabarica* possesses significant anticancer properties.

Key words: *Anisomeles malabarica*, Labiatae, diethylnitrosamine, anticancer, ethanolic leaf extract

INTRODUCTION

Among various diseases attributed to mortality in humans all over the world, cancer is a leading cause. Dietary factors continue to play a complex and multifaceted role in the aetiology of cancer. Apart from cigarette smoking and chronic inflammation and infection, nutrition accounts for up to one third of the total cause of cancer (Sugimura, 2002). Cancers most commonly associated with diet include esophageal, stomach, colon, liver and the prostate.

Cancer is one of the most dreaded diseases of the 20th century and spreading further with continuance and increasing incidence in 21st century. In the United States, as the leading cause of death, it accounts for 25% of all the deaths in humans presently. It is considered as an adversary of modernization and advanced pattern of socio-cultural life dominated by Western medicine. Multidisciplinary scientific investigations are making best efforts to combat this disease, but the sure-shot, perfect cure is yet to be brought into world medicine (Premalatha and Rajgopal, 2005).

DEN is considered to be a genotoxic carcinogen (Lewis *et al*., 1997). It is often assumed that DEN initiates and propagates tumor development primary by inducing DNA alterations that lead to mutations (Peto *et al*., 1991). Indeed, indicative mutations in the *ras* gene have been observed in mouse liver tumors arising in response to DEN treatment (Stowers *et al*., 1988).

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Since the increase in the use of synthetic chemicals in cancer therapy has led to many side effects and undesirable hazards, there is a worldwide trend to go back to natural resources (medicinal plants) which are therapeutically effective, culturally acceptable and economically within the research the poor people (Fauziah *et al*., 2005). Medicinal plants have become a major component of human health care as they have no or less side effects. Surveys conducted in Australia and US indicate that almost 48.5 and 34% of respondents had used at least one form of unconventional therapy including herbal medicine (Edease, 2000).

Over the centuries no fewer than 3000 plants species have been used to treat cancer (Lewis and Elvin, 1997). Many new plants were studied to identify natural cancer chemotherapeutic agents (Rates, 2001). *Anisomeles malabarica* (L.) R. Br. (Labiatae), distributed throughout India, which is commonly known as Aruvaachadachi. A number of pharmacological effects of *Anisomeles malabarica* have been reported by Chopra *et al*. (1956). The herb is reported to possess anticancer, antispasmodic, diaphoretic, antipyretic and antiperiodic properties (Varier, 1994). The plant decoction or leaves essential oil used externally in rheumatism (Chopra *et al*., 1956). In the present study, an attempt has been made to evaluate anticancer potential of *Anisomeles malabarica* (L.) R.Br. ethanolic leaf extract on DEN induced Albino mice.

MATERIALS AND METHODS

Plant Materials

The leaf of *Anisomeles malabarica* were collected from river bank of Tiruchirappalli district, (India) in the month of January 2006. The identity of plant was verified at Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, India. The plant material was dried in air and then milled to a fine powder of 1mm mesh size as described by Antonio and Britto (1998).

Extraction Procedure

The dried and powdered plant materials (100 g) were extracted successively with 600 mL of Ethanol (1:6 w/v) by using soxhlet extractor for 48 h at a temperature not exceeding the boiling point of the solvent (Lin *et al*., 1999). The extracts were filtered using Whatman No.1 filter paper and then concentrated in vacuum at 40°C using a Rotary evaporator. Each extracts transferred to glass vials and kept at 4°C before use.

Animals

Male wistar albino Mice (25-30 g) were used. The animals were housed in polypropylene cages with sterile, inert husk materials as bedding. The experimental animal were maintained under controlled environment conditions of light and dark cycle (light 12 h: dark 12 h, temperature 23±2°C and relative humidity 55±10%). Animals were allowed to take standard laboratory feed and tap water.

Experimental Groups and Protocol

Prior to start of the experiment, all animals were caged and acclimatized for one week period. In total, 20 mice with body weights ranging from 25±1.9 to 27±2.1 g were used in the experiment and were divided into 4 groups. Group-I served as control. Carcinogenesis was induced in the groups II and III by administrating intraperitoneally 50 mg kg^{-1} of DEN (in saline) once in a week for a period of three week. Soon after DEN administration, EAM (100 mg kg^{-1}) were orally given to group III and group IV was administrated EAM alone.

Enzyme Assays

After the experimental period, blood from the animals was collected by cardiac puncture and centrifuged at 5000 rpm for 10 min the serum was stored at -70°C for further analysis. All surviving

mice were sacrificed exsanguification from the abdominal aorta under light anesthetic condition at the end the day. Liver was dissected and 1 g was homogenized in phosphate buffer saline (PBS 0.05M, pH 7.4). The homogenate was centrifuged at 5000 rpm for 15 min at 4°C.

The supernatant thus obtained was used for biochemical analysis. Biochemical assessments were performed with the supernatant of liver and serum total protein concentration was determined by the method of Lowry *et al*. (1951). Glutamate Pyruvate Transaminase (GPT) and Glutamate Oxaloacetate Transaminase (GOT) activities were assayed as previously described (Mohun and Cook, 1957). Acid Phosphatase (ACP) and Alkaline Phosphatase (ALP) activities were measured by the methods of King and Armstrong (1934).

Histopathological Study

The animals were sacrificed and the abdomen was cut open to remove the liver. The liver was fixed in Bouin s solution (mixture of 75 mL of saturated picric acid, 25 mL of 40% formaldehyde and 5 mL of glacial acetic acid) for 12 h, then embedded in paraffin using conventional methods (Galighor and Kozloff, 1976) and cut into 5 µm thick sections and stained using haematoxylin-eosin dye and finally mounted in di-phenyl xylene. Then the sections were observed under microscope for histopathological changes in liver architecture.

Statistical Analysis

Results were expressed as mean±SE the intergroup variation was measured by one way analysis (ANOVA). Statistical significance was considered at $p<0.05$. The statistical analysis was done using the SPSS software version 13.

RESULTS AND DISCUSSION

DEN intoxicated in normal mice elevated the level of liver Protein, GOT, GPT, ACP and ALP were observed significantly indicating acute hepato cellular damage when compared with control. The mice treated with ethanolic leaf extract of *Anisomeles malabarica*, showed a significantly ($p<0.05$) decrease in all the elevated liver protein, GOT, GPT, ACP and ALP levels (Table 1).

DEN induced cancerous mice, significantly ($p<0.05$) increase in serum total protein, SGPT, SGOT, ACP and ALP in mice were found to be 3.63±0.02 mg dL^{-1}, 49.31±0.02, 112.12±0.07, 191.25±0.14 and 172.29±0.25 IU L^{-1}, respectively (Table 2). Whereas animals treated with ethanolic

Table 1: Effect of pretreated with ethanolic leaf extract of *Anisomeles malabarica* on liver tissue enzyme activity of DEN induced cancer in mice

Groups	Protein (mg/100 g)	SGPT (IU L^{-1})	SGOT (IU L^{-1})	ACP (IU L^{-1})	ALP (IU L^{-1})
Normal	768.90±0.27	99.45±0.22	49.47±0.10	53.91±0.43	219.26±0.26
DEN alone	832.18±0.42	262.17±0.54	101.84±0.61	78.92±0.23	297.88±0.16
DEN+EAM	783.33±0.23*	128.32±0.98*	56.92±0.49*	61.86±0.36*	243.26±0.05*
EAM	763.47±0.98	108.84±0.98	49.32±0.23	56.65±0.15	217.63±0.30

Each value represent mean±SE, No. = 5, *: Significant reduction compared to DEN inducted Mice at $p<0.05$ level

Table 2: Effect of pretreated with ethanolic leaf extract of *Anisomeles malabarica* on serum enzyme activity of DEN induced cancer in mice

Groups	Protein (mg/100 g)	SGPT (IU L^{-1})	SGOT (IU L^{-1})	ACP (IU L^{-1})	ALP (IU L^{-1})
Normal	3.63±0.02	49.31±0.02	112.12±0.07	191.25±0.14	172.29±0.25
DEN alone	5.26±0.12	78.39±0.14	169.24±0.20	227.62±0.16	244.33±0.62
DEN+EAM	4.67±0.15*	53.25±0.06*	129.30±0.18*	203.51±0.19*	195.93±0.46*
EAM	4.19±0.20	50.81±0.38	113.39±0.22	192.31±1.01	168.91±0.25

Each value represent mean±SE, No. = 5, *: Significant reduction compared to DEN inducted Mice at $p<0.05$ level

leaf extract of *Anisomeles malabarica* exhibited a decreased in total protein (4.67±0.15 m g dL^{-1}), SGPT (53.25±0.06 IU L^{-1}), SGOT (129. 30±0.18 IU L^{-1}), ACP (203.51±0.19 IU L^{-1}) and ALP (195.93±0.46 IU L^{-1}) in DEN induced liver cancer in mice.

Histopathological examination of liver sections of control group showed normal cellular architecture (Fig. 1a). Disarrangement of normal hepatic cells with intense centrilobular necrosis were observed in DEN intoxicated liver (Fig. 1b). The mice treated with ethanolic leaf extract of *Anisomeles malabarica* and intoxicated with DEN, showed less vacuole formation, fatty changes and absence of necrosis (Fig. 1c). Ethanolic extract alone treated mice showed (Fig. 1d), overall no visible changes and normal histology were observed.

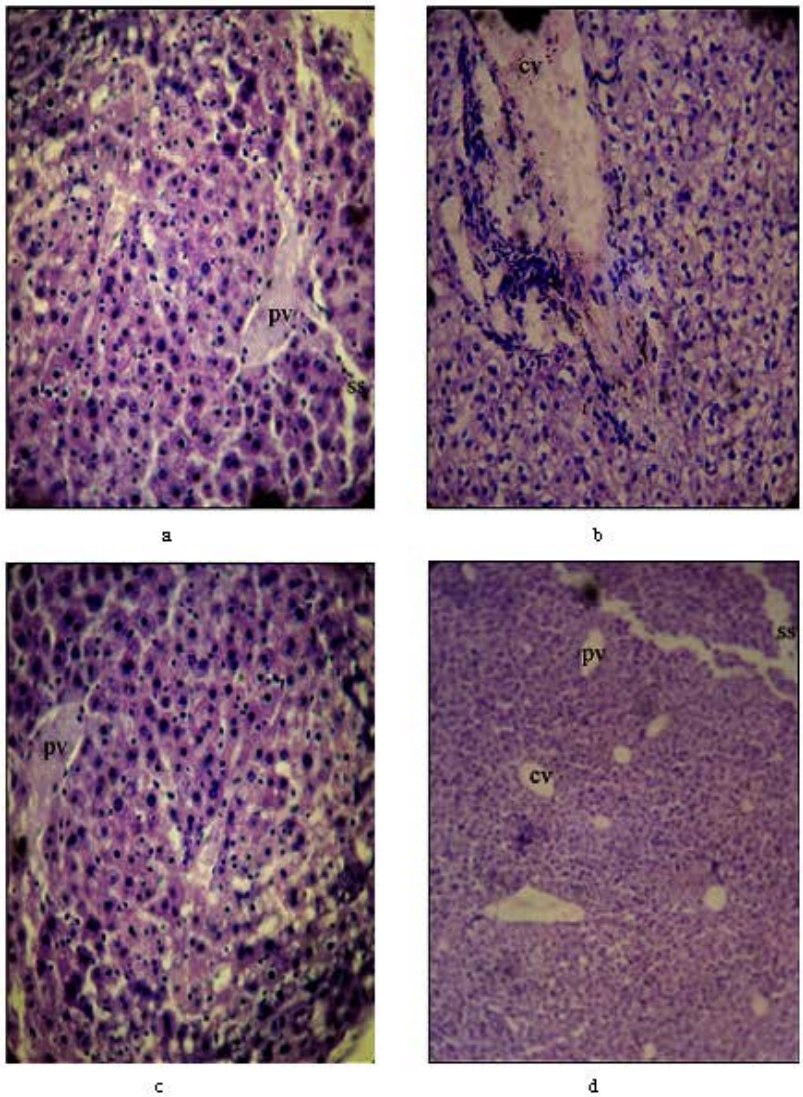

Fig. 1: Photomicrograph showing the effect of crude ethanolic leaf extract of *Anisomeles malabarica* (L.) R. Br. 1a) normal control liver (group I), 1b) DEN induced cancer liver (group II), 1c) DEN induced cancer treated with plant extract liver (group III), 1d) plant extract alone liver (group IV). cv: Central vein; pv: Portal vein; ss: Sinusoidal space

DISCUSSION

In Indian system of medicine certain herbs are claimed to provide relief against liver disorders. Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with current chemotherapeutic agents. One of the more versatile plants used as a ethanolic extract of leaves of *Anisomelos malabarica*, a member of the Labiatae family was taken for the anticancer evaluation in DEN induced mice.

Elevation of the plasma levels of cytoplasmic and mitochondrial enzymes is a sensitivity indicator of liver Damage (Sherlock and Dooley, 1993). Drug induced liver damage has been reported to correlate with an increase in the activity of these enzymes (Rhoades and Pflanzer, 1996). Among the various phosphatases, ACP and ALP have attained much attention because of their location in the plasma membrane and possible role in active transport (Mehendale *et al*., 1994). The efficacy of any hepatoprotective drug is essentially dependent on its capability of either reducing the harmful effects of a hepatotoxin or of maintaining the normal physiological mechanisms that are unbalanced by a hepatotoxin.

Elevation of serum ACP, ALP, GOT and GPT is a known effect of DEN toxicity which specifically affects the liver (Anand *et al*., 1992) and activities of ACP, ALP, GOT and GPT are most commonly used biochemical makers of liver damage (Sturgill and Lambert, 1997). They reported all the serum parameters ACP, ALP, GOT and GPT were reduced significantly when the mice were pretreated with extracts. These results were similar to the results reported in this current research using DEN as cancer toxicants. Anticancer effect of *Anisomelos malabarica* leaf was observed in DEN induced liver cancer in mice. The present investigation reemphasizes the usefulness of *Anisomelos malabarica* in traditional medicine as an anticancer agent used in traditional medicine.

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REFERENCES

Anand, K.K., B. Signh, D. Chend and B.K. Bhandan, 1992. An evaluation of *Lawsonia alba* extract as hepatoprotective agent. Planta Medica, 58: 22-25.

Antonio, M.A. and A.R. Souza Britto, 1998. Oral anti inflammatory and anti ulcerogenic activities of a hydroalcoholic extract and partitioned fraction of *Turnera ulmifora*. J. Ethnopharmacol., 61: 215-228.

Chopra, R.N., S.L. Nayar and I.C. Chopra, 1956. Glossary Indian Medicinal Plants. Council of Scientific and Industrial Research, New Delhi, pp: 19.

Edease, M., 2000. Superoxide Dismutase: Recent Advances, Clinical and Nutritional Application. In: Proceedings of the 2nd International Conference, Institute Pasteur, Paris, pp: 18-19.

Fauziah, O., P. Hanachi, S. Yogespiriya and R. Asmah, 2005. Reducing effect of *Strobilanthes crispus* leaf extract in hepatocarcinogenesis rats. Int. J. Cancer Res., 1: 109-112.

Galighor, A.E. and E.N. Kozloff, 1976. Essential of Practical Micro Technique. 2nd Edn., Lea and Febiger, New York, pp: 210.

King and Armstrong, 1934. Convenient method for determining serum and bile phosphatese activity. Cancer Med. Assoc. J., 31: 376.

Lewis, D.F.V., P.G. Brantom, C. Ioannides, R. Walker and D.V. Parke, 1997. Nitrosamine carcinogenesis: Rodent assays, quantitative structure activity relationships and human risk assessment. Drug Metab. Rev., 29: 1055-1078.

Lewis, W.H. and M.P.F. Elvin-Lewis, 1997. Plants Affecting Man's Health. In: Medicinal Botany. Wiley, A. (Ed.), Interscience Publication, New York, pp: 105-148.

Lin, J., W.A.R. Opak and M. Geheeb-Keller, 1999. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and antimicrobial activities. J. Ethnopharmacol., 68: 267-274.

Lowry, O.M., N.J. Rosebrough, A.L. Farr and R.J.L. Randall, 1951. Protein measurement with folin phenol reagent. J. Biol. Chem., 193: 265-275.

Mehendale, H.M., R.A. Roth, A.J. Gandolfi, J.E. Klaunig, J.J. Lemasters and L.R. Curtis, 1994. Novel mechanisms in chemically induced hepatotoxicity. Faseb. J., 8: 1285-1295.

Mohun, A.F. and P. Cook, 1957. Simple methods for measuring serum levels of the Glutamateoxaloacetate and glutamic-pyruvate transaminase in routine laboratory. J. Clin. Pathol., 10: 374-399.

Peto, R., R. Gray, P. Brantom and P. Grasso, 1991. Effects on 4080 rats of chronic ingestion of N-nitrosodiethylamine or N-nitrosodimethylamine: A detailed dose-response study. Cancer Res., 51: 6415-6451.

Premalatha, B. and R. Govindarajan, 2005. Cancer an ayurvedic perspective. Pharmacol. Res., 51: 19-30.

Rates, S.M.K., 2001. Plants as source of drugs. Toxicon, 39: 603-613.

Rhoades, R. and R. Pflanzer, 1996. Human Physiology. Saunders College Publishing, London.

Sherlock, S. and J. Dooley, 1993. Disease of the Liver and Billiary System. 9th Edn., Blackwell Scientific Publishing, London.

Stowers, S.J., R. Wiseman, J. Ward, E. Miller, J. Miller, M. Anderson and A. Eva, 1988. Detection of activated proto-oncogenes in N-nitrosodiethylamine-induced liver tumors: A comparison between B6C3F1 mice and Fischer 344 rats. Carcinogenesis, 9: 271-276.

Sturgill, M.G. and G.H. Lambart, 1997. Xenobiotic-induced hepatotoxicity: Mechanisms of liver injury and methods of monitoring hepatic function. Clin. Chem., 43: 1512-1526.

Sugimura, T., 2002. Food and Cancer. Toxicology, 182: 17-21.

Varier's, P.S., 1994. Indian Medicinal Plants, a Compendium of 500 species. Orient Longman Ltd., Madras, pp: 157.