Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2013, 5 (6):105-114
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Carcinoprevention by *Indigofera aspalathoides* against 20 Methylcholanthrene induced fibrosarcoma in Wistar strain albino rats.

[1#]Selva Kumar Sivagnanam, [1]Mudiganti Ram Krishna Rao and [2]Maruthaiveeran Periyasamy Balasubramanian

[1]Department of Industrial Biotechnology, Bharath University, Chennai, India
[2]Department of Pharmacology and Environmental Toxicology, Dr. A. L. M. Post graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai, India

---

ABSTRACT

The present study was undertaken to assess the carcinopreventive properties of aqueous extracts of an Indian medicinal plant Indigofera aspalathoides, on 20-Methylcholanthrene induced fibrosarcoma in Wistar strain male albino rats. Fibrosarcoma was induced by subcutaneous implantation of Millipore filter disc impregnated with 5% suspension of 20- Methylcholanthrene in paraffin oil and the tumors appeared in about six weeks. The fibrosarcoma was isolated and proved by histo pathological examinations. Intraperitoneous (i. p.) administration of 250 mg/kg b.w. /day of aqueous extract of Indigofera aspalathoides for 30 days was conducted. The aqueous extract of Indigofera aspalathoides treatment reduced the tumor size and volume in control and experimental animals. The levels of electrolytes such as Na^+, K^+, Ca^{2+}, Mg^{2+} were analyzed in serum of control and experimental animals and the Na^+, K^+ ATPases, Ca^{2+}ATP ase and Mg^{2+} ATPases levels were observed in erythrocyte membrane and liver and kidney of control and experimental animals. The levels of these electrolytes and those of the enzymes were altered significantly in control and experimental animals. Our results clearly indicated the carcinopreventive properties of Indigofera aspalathoides on 20-ethylcholanthrene induced fibrosarcoma in rats.

Key Words: Carcinoprevention, Cycloxygenese, Fibrosarcoma, *Indigofera aspalathoides*, Lipoxygense.

INTRODUCTION

Cancer is a dreaded disease posing a challenge to the rank and file of researchers and medical professionals. Even though a large amount of work has been undertaken at various levels of research, the perfect cure to this disease is still elusive. Due to the metastatic nature of the disease radiation and surgery have often led to failures. Thus chemotherapy is by far the best available mode of treatment for cancer. A large number of chemopreventive agents have been identified in epidemiological and experimental studies. The goal of chemoprevention can be accomplished at three different levels, 1. At the clinical level, the development of cancer must be prevented or delayed, 2. At the tissue level, progressive or pre-malignant lesions must be suppressed or reversed and 3. At the cellular level, atypical clones must be eradicated [1]. All the above processes are achieved by a combination of medicines such as Vincristine and Vinblastine and chemicals such as Cis-platin. Many new medicines both synthetic as well as plant derived are being added to the list in controlling various cancers. Chemotherapy, although being the best way of treatment, is beset with the major disadvantage in its undesirable toxic side effects. The toxic side effects produced by some of these chemotherapeutic agents have limited their extensive use. Thus searching for

alternative chemotherapeutic agents which do cure cancer and at the same time have minimum or no side effects could be a practical strategy. An alternative solution of this problem is the use of medicinal plant preparation to arrest the insidious character of the disease. In China, traditional Chinese medicinal herbs are used in the effective treatment against most of the tumors [2]. In all the three Indian systems of medicine, Ayurveda, Siddha and Unani, many medicinal plants are widely used to treat various diseases, including tumors. Several epidemiologists have highlighted the role of plants and plant components, in reducing the risk of cancer in a variety of tissue and organs. Herbal medicines play a vital role in the treatment of patients with soft tissue tumors [3]. Plants and plant products are used to treat various diseases including cancers because of the low or no toxicity [4, 5]. In the present study, one herb known as *Indigofera aspalathoides* was chosen to evaluate its role as an antineoplastic agent. The plant *Indigofera aspalathoides* Vahl. belonging to family Papilionaceae, is a low under shrub with copious terete branches. It is found in South India and Sri Lanka [6]. It is found to be active against transplantable tumors and inflammations [7].The leaves, flowers and tender shoots are said to be cooling and demulcent and are used to treat leprosy, syphilis and skin disorders. This is one of the important ingredients of the specific oil for syphilitic and other skin diseases. The aqueous extract of *Indigofera aspalathoides* contain mainly alkalaoids, flavonoids, saponins, tannins, steroids and reducing sugars which have the ability to counteract the adverse biological effects of carcinogen.

The scope of the present study was to use the crude aqueous extract of the plant for the treatment of fibrosarcoma in continuance of our work reported earlier by us [8, 9, 10, 11] at a holistic level. Many active principles from plants like Vincristine are already in use but with many side effects. The reason for the treatment with a crude extract was to go for a holistic approach presumably to avoid any side effects, since along with the main ingredient which cures the disease other minor components might be giving a synergistic effect or might be reducing the side effects of the main constituent.

20-methylcholanthrene is a polycyclic hydrocarbon available in the environment due to the combustion of carbon products. This is one of the major carcinogens to humans and animals. The current study focusses on the promising antitumor potency of the aqueous extracts of *Indigofera aspalathoides* on 20-Methylcholanthrene induced fibrosarcoma in rats.

MATERIALS AND METHODS

2.1 Plant Materials

Fresh aerial parts (leaves, stems, seeds and flowers) of the plant *Indigofera aspalathoides* were obtained, identified and authenticated by the Chief Botanist, Tamil Nadu Aromatic and Medicinal Plants Corporation Limited (TAMPCOL), at Government Siddha Medical College Campus, Arumbakkam, Chennai, India.

2.2 Preparation of Plant extract

1 kg. of the shade dried and coarsely powdered aerial parts of the plant *Indigofera aspalathoides* was charged in an aspiration bottle and allowed to soak in double distilled water for 48 hrs at room temperature. The extract was filtered and concentrated on a water bath. The inorganic material was precipitated and filtered off. The filtrate was again concentrated in a China dish and dried in vacuum. The yield of the extract was 10% w/w of the powdered aqueous extract. This was stored in refrigerator for further and future use.

2.3 Phytochemical Analysis of Indigofera aspalathoides

The extracts were analyzed for the presence of alkaloids, terpenoids, reducing sugars, saponins, tannins, carbonyls, flavonoids, phlobatannis and steroids [12, 13, 14].

2.3 a. Test for Alkaloids

About 0.2 gm of plant extract in a test tube was warmed with 2% Sulphuric acid for 2 minutes. It was filtered and few drops of Dragondorff's reagent were added and observed for the presence of orange red precipitates for the presence of alkaloids.

2.3 b. Test for Cardiac glycoside
Keller-Killani Test
About 0.5 gm of plant extract was taken in a test tube with 2 ml of glacial acetic acid containing a drop of ferric chloride solution. This was under layered with 1 ml of concentrated tetra oxo sulphate (VI) acid. Observation of brown ring formation at the interface would indicate presence of cardiac glycosides [15].

2.3 c Test for Terpenoids
About 0.5 g plant extract was taken in a test tube with 2 ml of chloroform. To this, concentrated Sulphuric acid was added carefully to form a layer. The presence of reddish brown color at the interface would show positive results for the presence of terpenoids.

2.3 d Test for Reducing sugars
2 ml of crude plant extract was added with 5 ml of distilled water and filtered. The filtrate was boiled with 3-4 drops of Fehling's solution A and B for 2 minutes. Appearance of orange red precipitate would indicate the presence of reducing sugars.

2.3 e Test for Saponins
About 0.2 gm of plant extract was taken in a test tube and added with 5 ml of distilled water. This solution was boiled. Observation for the occurrence of frothing (appearance of creamy mass of small bubbles) would indicate the presence of Saponin.

2.3 f Test for Tannin
A small quantity of plant extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate and observed for dark green solution that would indicate the presence of tannin.
2.3 g Test for Carbonyl group 2 ml of plant extract was taken in a test tube and few drops 2, 4,-di nitro phenyl hydrazine solution was added and shaken. Instant appearance of yellow crystals would indicate the presence of an aldehyde (Carbonyl group).

2.3 h. Test for Flavonoids
About 0.2 gm plant extract was taken in a test tube and mixed with diluted Sodium hydroxide. To this diluted Hydrochloric acid was added. Observation of yellow solutions that turn colorless later, would indicate the presence of flavonoids.

2.3 i Test for Phlobatanin
About 0.5 gm of plant extract is taken in a test tube and dissolved in distilled water and filtered.

The filtrate was boiled with 2% Hydrochloric acid solution. Observation of a red precipitate indicates the presence of Phlobatanin.

2.3 j Test for Steroids
0.5 gm of ethanolic extract of each sample was mixed with 2 ml of acetic anhydride and each sample with 2 ml of Sulphuric acid .Observe for the color change from violet to blue or green insamples would indicate the presence of steroids.

2.4 Acute toxicity Studies
Acute toxicity study of AEIA was done as per OECD guideline 425 using albino male rats. The animals were kept fasting overnight providing only water, after which the extract was administered orally for one animal at the limit dose of 2500 mg kg^{-1} and observed for 14 days (special attention for the first 4 hrs of administration followed by the next 20 hrs). In case of the death, the limit test was terminated and main test was conducted. If the animal survived, four additional animals were dosed sequentially so that five animals could be tested. However, if three animals died, the limit test was terminated and the main test was performed. The LD_{50} is greater than 2500 mg kg^{-1} if three or more animals survived. If an animal died unexpectedly late in the study and there were other survivors, it was appropriate to stop dosing and observing all animals to see if other animals also die during a similar observation period.

2.5 Acute toxicity test

The AEIA has not shown any mortality at the limit dose of 2500 $mgkg^{-1}$ body weight. AEIA was found to be safe even at a higher concentration and based on this, the dose for the chemo-preventive activity was chosen.

2.6 Animals

Wister strain male albino rats weighing 100 to 120 gm, were obtained from TANUVAS-LAMU, Madhavaram, Chennai, India. The animals were fed with normal pellet diet (rat chew) and water *ad libitum*. The study protocol, approved by the Ministry of Social Justice and Empowerment, Government of India, was followed [Institutional Animals Ethics Committee (IAEC) number 07/15/02].

2.7 Sample Collection

The animals were sacrificed by cervical decapitation at the end of the experimental period and blood was collected to separate serum for bio chemical analysis. The liver and kidney were dissected out and known weight of liver and kidney were homogenized in 0.1M Tris- HCl buffer (pH- 7.4). Animals were starved overnight before sacrifice.

2.8 Chemicals

All the chemicals and reagents used were purchased from M/s. Sigma Chemicals, USA.

2.9 Induction of Fibrosarcoma

Fibrosarcoma was induced in Wister strain of male albino rats by subcutaneous implantation of Millipore filter disc, impregnated with 5% suspension of 20- MCA in paraffin oil [16]. Tumors which appeared in about 4 weeks after implantation were, highly localized and were maintained by serial transplantation. The tumor was minced and suspended in normal saline. A suspension of about $1x10^6$ cells in 0.5 ml of saline was injected subcutaneously into the thigh. The transplanted tumor became palpable in 4-6 days time and became measurable on 9^{th} day. It grew up steadily till the end of second week, after which necrosis set in and animal eventually died in about 4 weeks.

2.10 Histopathological Analysis

In group I animals, the liver sections of control animals showed the normal architecture of the hepatic cells with well preserved cytoplasm. In Group II rats the liver sections of fibrosarcoma bearing animals showed mild congestion of sinusoids with central dilation. In group III the liver sections of fibrosarcoma bearing animals treated with *Indigofera aspalathoides* show mild congestion of the sinusoids without central dilation. In group IV drug alone treated animals the liver sections showed near normal architecture of the tissue. In group I the kidney sections of control animals show the normal architecture of the renal cells with regular histology. In group II animals the kidney sections show tubular dialation with congested vessels. In Group III animals showed near normal kidney histology. In group IV drug alone treated animals the kidney showed normal architecture. These results were published elsewhere (Selva Kumar *et al*, 2012).

2.11 Experimental Design

The rats were divided into four different groups each group consisting of six animals. Group I animals served as normal control animals, Group II animals were fibrosarcoma bearing animals, Group III animals were fibrosarcoma bearing animals treated with aqueous extract of *Indigofera aspalathoides* intraperiotonially at a dose of 250 mg/kg b. w. /day for 30 days and Group IV animals were administered with the aqueous extract of *Indigofera aspalathoides* alone, at a dose of 250 mg/kg b. w./ day for 30 days, served as drug control animals.

2.12 Tumor Measurements

Tumor measurements were made using a vernier calipers and tumor diameter (Td) was calculated using the formula stated elsewhere. The experiments were repeated twice.

$$Td\ (cm) = \frac{\text{Length of tumor (cm)} + \text{Width of tumor (cm)}}{2}$$

2.13 Biochemical Estimations

2.13 a Estimation of Sodium and Potassium

Preparation of Standard and Working Standard solution

Sodium and Potassium quantities were determined on a diluted aliquot of sample solution by using flame photometry. 2.2919 gm of Sodium carbonate and 1.85 gm of Potassium sulphate was weighed accurately and

dissolved in 200 ml standard flask using demonized water. This solution contained 2000 ppm each of sodium and Potassium. The working standard consisted of the above stock solution were diluted to give the concentration from 10 ppm to 50 ppm and were used as working standard.

Procedure

A known concentration of sodium and potassium solutions was used as standards. Demonized water was used to set zero. The fluid analyte was sprayed as a fine mist into a non luminous flame which becomes colored according to the characteristic emission of sodium and potassium present in the fluid. The flame was simultaneously monitored by both sodium and potassium channels. A dilution correction was made for the tissue and serum sodium and potassium concentrations with the known standards concentrations of sodium and potassium.

2.13 b Estimation of Calcium and Magnesium

Calcium and Magnesium contents were estimated by atomic absorption spectrometry (Perklin-Elmer Model 2380). (Table 4)

2.13 c Estimation of Adenosine Triphosphatase

Adenosine triphosphatase enzyme catalyses the conversion of Adenosine Triphosphate into Adenosine Diphosphate. During the conversion 1 mol of phosphorus in liberated. The inorganic phosphorus is estimated according to the method of Fiske and Subbarow (1925) [17]. The proteins are precipitated with trichloroacetic acid. The free filtrate reacts with acid molybdate solution to form phosphomolybdic acid which is reduced by the addition of 1-Amino 2-napthol-4-sulphuric acid (ANSA) to produce blue colour. The intensity of the colour produced was proportional to the amount of phosphorus present.

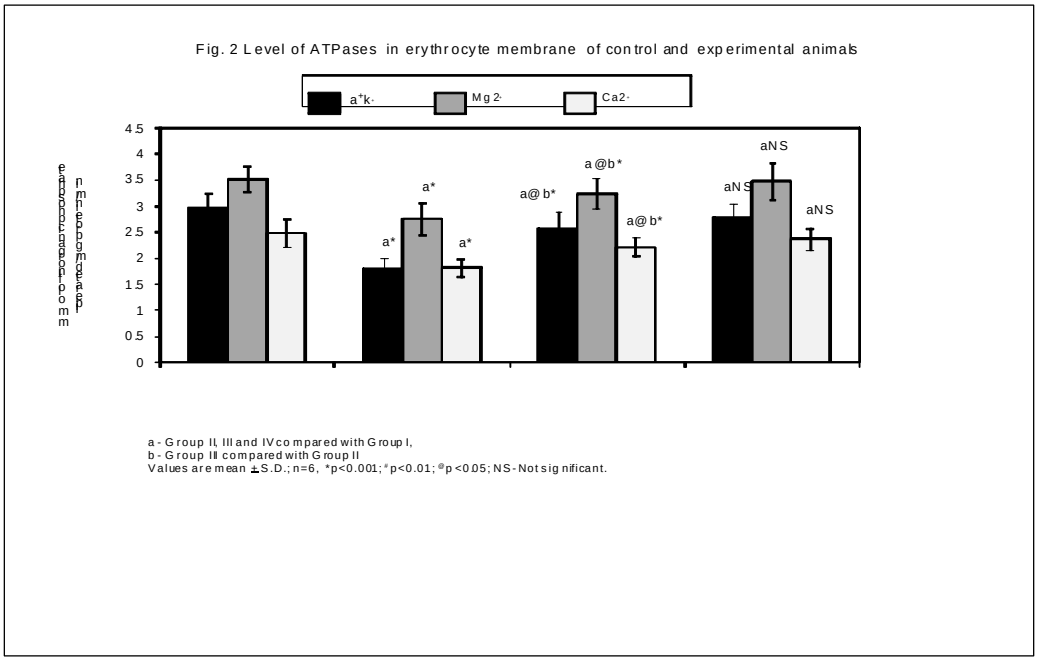

Figure 1 shows the levels of electrolytes such as Na^+, K^+, Ca^{2+} and Mg^{2+} in serum of control and experimental animals. The Na^+, K^+ and Ca^{2+} levels were elevated, whereas that of Mg^{2+} was reduced in group II fibrosarcoma bearing animals. The levels of electrolytes were reversed to near normal in Group III fibrosarcoma bearing *Indigofera aspalathoides* treated animals. No marked changes were observed in *Indigofera aspalathoides* alone treated Group IV animals.

2.13 d Isolation of erythrocyte and its membrane

The blood was collected with 3.7% Trisodium citrate as anticoagulant (0.1 ml), was used for the erythrocyte isolation. Plasma was separated by centrifugation at 2000g for 20 min. The packed cells were washed thrice with physiological saline and the plasma free red cells were used for analysis. The erythrocyte membrane was isolated according to the method of Dodge *et al*, 1963 with a change in buffer, according to the method of Quist, 1980)[18, 19]. Ca^{2+} ATPase levels were estimated by the method of Hjerton and Pan 1983, Na^+/K^+ - ATPase was estimated by

the method of Bonting, 1970 and that of Mg^{2+} ATPase was assayed by the method of Ohnishi *et al*, 1962[20, 21, 22]. Total protein was estimated by the method of Lowry *et al*, 1951[23].

2.14 Statistical Analysis

The data presented were as mean ± standard deviation (SD). One way analysis of variance was (ANOVA) was performed. The Tukey's multiple comparison method was used to compare the means of different groups and the significance was denoted. Different significance values were denoted by p value. All these analyses were carried out using the statistical package for Social Science version 7.5.

RESULT

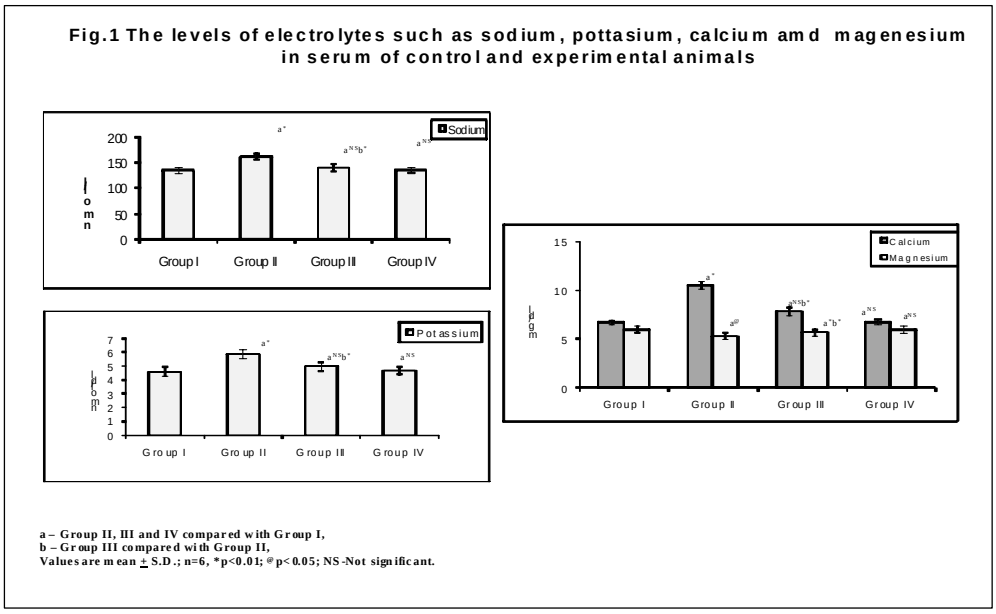

Figure 2 shows the levels of ATPase in erythrocyte membrane of control and treated animals. In fibrosarcoma bearing animals the levels of Na^+ and K^+ ATPases, Mg^{2+} ATPases and Ca^{2+} ATPase were significantly decreased (p<0.001) when compared to normal control animals. These enzyme levels normalized after the treatment of *Indigofera aspalathoides* in fibrosarcoma bearing animals. No significant alterations were noticed in Group IV animals treated with the plant extract alone.

Table 1 represents the activities of Adenosine Triphosphatases such as Na^+, K^+, ATPases, Mg^{2+} ATPases and Ca^{2+} ATPases in the liver of control and experimental animals. A significant decrease (p<0.001) in the levels of Na^+, K^+, Mg^{2+} ATPases was observed where as there was an increase in the Ca^{2+} ATPases levels in the fibrosarcoma bearing animals. After the administration of *Indigofera aspalathoides*, these enzyme levels reversed to near normal condition in group III animals except for Ca^{2+} ATPase activity which showed only moderate decrease after the treatment. No alteration was observed in Group IV *Indigofera aspalathoides* alone treated animals.

Table 1 The Activities of Adenosine Triphosphatases in liver of Control and Experimental animals

Parameters (µ mol of Inorganic Phosphate liberated/mg proptein/min.)	Group I	Group II	Group III	Group IV
Na^+, K^+ –ATPase	4.22 ± 0.11	2.40 ± 0.13^{a*}	3.26 ± 0.34$^{aNS\ b*}$	4.20 ± 0.12^{aNS}
Mg^{2+} – ATPase	1.47± 0.09	0.91 ± 0.11^{a*}	1.22 ± 0.14$^{a@\ b*}$	1.50 ± 0.08^{aNS}
Ca^{2+} –ATPase	2.36 ± 0.21	3.21 ± 0.16^{a*}	2.31 ± 0.16$^{aNS\ b*}$	2.39 ± 0.06^{aNS}

a- Group II, Group III and Group IV compared with Group I.; b- Group III compared with Group II.
*Values are mean ±S.D., n = 6.; *- p< 0.001, @- p<0.05, NS- Not Significant*

Table 2 shows the levels of ATPases in kidney of control and experimental animals. A significant decrease ($p<0.001$) in the levels of Na^+, K^+ and Mg^{2+} ATPases were observed in kidney of fibrosarcoma bearing group II animals whereas the Ca^{2+} ATPases levels were increased significantly. It was observed that all the enzymes reached near normal levels in fibrosarcoma bearing *Indigofera aspalathoides* treated Group III animals. There was no significant change in these levels in *Indigofera asplathoides* alone treated animals.

Table 2. The Activities of Adenosine Triphosphatases in kidney of Control and Expertimental animals

Parameters (µ mol of Inorganic Phosphate liberated/mg proptein/min.)	Group I	Group II	Group III	Group IV
Na^+, K^+ –ATPase	2.54 ± 0.13	1.86 ± 0.21^{a*}	2.48 ± 0.14$^{aNS\ b*}$	2.60 ± 0.18^{aNS}
Mg^{2+} – ATPase	1.58± 0.17	0.66 ± 0.08^{a*}	1.26 ± 0.13$^{a@\ b*}$	1.48 ± 0.21^{aNS}
Ca^{2+} –ATPase	2.40 ± 0.07	3.50 ± 0.15^{a*}	3.42 ± 0.24$^{aNS\ b*}$	2.42 ± 0.12^{aNS}

a- Group II, Group III and Group IV compared with Group I.; b- Group III compared with Group II.
*Values are mean ±S.D., n = 6.; *- p< 0.001, @- p<0.05, NS- Not Significant*

Table 3 depicts the phytochemical constituents of *Indigofera aspalathoides.*

Table-3. The results of phytochemical analysis of *Indigofera aspalathoides* aqueous extract.

Test Parameters									
Alkalioid	Cardiac Glycosides	Terpenoides	Reducing Sugars	Saponins	Tannins	Carbonyl	Flavonoids	Phlobatinis	Steroids
+	-	+	+	+	+	-	+	-	+

Key + = Positive; - = Negative

DISCUSSION

20 Methylcholanthrene causes a wide range of tumors in all animal species. This compound belongs to polycyclic aromatic hydrocarbons, PAHs, are ubiquitous environmental agents commonly believed to significantly contribute to human as well as animal cancers. These chemicals are formed in the process of incomplete combustion of organic material and are found widely in the environment. The human exposure to PAHs is therefore unavoidable. Like many other carcinogens, PAHs are metabolized enzymatic ally to various metabolites of which some are reactive. In this process large group of enzymes are involved in the carcinogenic metabolism. 20-MCA has been used as an effective experimental model in the field of chemical carcinogenesis and cancer prevention. [24].

Chemoprevention, a science that has emerged during the last three decades, presents an alternative approach to reducing mortality from cancer. Chemopreventive interventions may be applied at any time during carcinogenesis, from the initial molecular defect through the accumulated molecular, cellular and histopathological aberrations that characterize the disease progression before an invasive and potentially metastatic stage [25]. The cancer chemoprevention aims at blocking, reversing or delaying carcinogenesis before the development of invasive disease by targeting key molecular derangements using pharmacological or nutritional agents.

Recently Selva Kumar *et al*, 2010 have also observed that the administration of aqueous extract of *Indigofera aspalathoides* (250 mg kg body wt. per day) was effective in preventive 20 MCA induced fibrosarcoma in rats. The authors have found that the there was a marked enhancement of the activities of Phase I enzymes such as NADPH-Cytochrome P_{450}, NADH Cytochrome b_5 Reductase and Aniline hydroxylase and Phase II enzymes such as Glutathion S transferase and UDP Glucronyl tranferase levels in *Indigofera aspalathoides* treated animals show the reduction in tumerogenesis. In addition to the impairment of dug metabolism, the decrease in the activities of these enzymes and the content GSH could impair the overall biotransformation process. The plant extract was found to act as a biofunctional inducer because it induces both Phase I and Phase II enzymes. Such induction may inhibit the formation of covalently bound complexes of 20 MCA with DNA, RNA and proteins and this in turn might cause inhibition of tumor process. The aqueous extract of *Indigofera aspalathoides* is also found to be an inducer of Phase II enzymes and hence it could be said that it acts a potential protective agent against 20 MCA induced fibrosarcoma. Due to the increased activity of GST the microsome mediated 20 MCA binding to DNA may be reduced in the

presence of *Indigofera aspalathoides* extracts. The anticancer activity of I A may be due to its ability in enhancing the activity of Phase I and Phase II enzymes [6].

The antioxidants have also been advocated to impart anticancer activity by several other mechanisms [26]. Trapping the ultimate carcinogen, modulating xenobiotic metabolizing enzymes, scavenging of free radicals, inhibiting generation of free radicals, inhibiting promotion stage of carcinogens by inhibiting cell proliferation through blocking of the Lipoxygenase/Cycloxygenase path way or by lowering Ornithine decarboxylase activity and by decreasing the bioavailability of ultimate carcinogen. The treatment with this plant showed protective action against Reactive Oxygen Species (ROS) induced by malignant tumor, possibly through its ability as an antioxidant in quenching superoxide anions or free radicals. It is also reported that the aqueous extract of *Indigofera aspalathoides* has shown the alteration of cell membrane glycoprotein synthesis and structure thus indicating its strong anticancer potency [27].

A large proportion of the world population especially in developing countries, depend on the traditional system of medicines for a variety of disease. Several hundred genera of plants are used medicinally, mainly as herbal preparations in the indigenous system of medicine in different countries and were the source of potent and powerful drugs which have stood the test of time and modern chemistry has not been able to replace most of them. Many pharmaceuticals that we use today are of botanical origin and are based on herbal remedies from folk medicines of native people. Most important drugs in the past 50 years or so were first isolated from the plants used ethnomedically. In fact, 74% of the biologically active plant derived compounds at present used were discovered as result of research on plant species first identified on ethnobotanical surveys [28]. Trace elements would extend their action directly or indirectly, on the carcinogenic process by affecting the permeability of cell membranes or by other mechanisms [29].

Statistically, significant differences from the normal distribution of Sodium, Potassium and Magnesium and other essential elements have been reported to occur in patients of various types of cancer. Fibrosarcoma bearing animals treated with anticancer agents like Cisplatin, showed as increase in Sodium and Potassium levels. Notachin *et al* 1994 reported that the Cispaltin treatment increases Sodium content in the renal cortex and decreases in the papilla, whereas Potassium content in renal cortex was found to be increased [30]. Mitochondrial injury is an important early event in anticancer agent toxicity to proximal tubule cells that precedes inhibition of Na^+, K^+ ATPase activity and loss of cell Potassium. Adenosine Triphosphatases are membrane bound enzyme or mostly occur on the baso-lateral membranes. Na^+, K^+ ATPase activity pumps Na^+ out of the cells. As a result the intracellular concentration of Sodium is lowered and an inward proton gradient develops and Na^+/ H^+ exchange is established across the brush borders of membrane which splits up the ATP for energy purpose. The activity of Na^+/K^+ ATPase can also be regulated by hormone proteins as 2^{nd} messengers. The lipid peroxidation was also associated with the inhibition of Na^+, K^+ ATPase activity in proximal tubule cell lysate and this occurs secondary to mitochondrial injury [31]. In our study also the levels of Na^+, K^+ ATPase activity in serum, liver and kidney were found to decrease significantly. Magnesium is mainly as intracellular cation involved in many enzymatic reactions. It is an important co factor for Adenosine Triphosphatases. Serum Magnesium concentration is maintained within a narrow range by the kidney and small intestine, since under conditions of Magnesium deprivation, both organs increase the fractional absorption of Magnesium. Magnesium excretion may be due to defective reabsorption process in the medullary nephrons or collecting ducts [32, 33]. Magnesium ATPase is distributed in adrenal cell components as they play a role in endergonic processes other than ion transport. The ion sensitive Mg^+ ATPases utilizes a pool of ATP that is not directly related to change in free energy for Sodium transport. It is poised to regulate the flow of potential energy from the mitochondria and from the cytoplasm. The Mg^{2+} ATPases activity increased significantly due to *Indogofera aspalathoides* treatment when compared with fibrosarcoma bearing animals.

Calcium is a major factor in converting reversible cell damage into irreversible cell injury and death [34]. An increase in Calcium levels can activate endogenous enzymes and allow it to initiate cell necrosis by interfering with production and utilization of energy [35]. It was also reported that the Ca^{2+} concentration increased significantly in kidney of tumor bearing rats [36]. The increase in renal endoplasmic reticulum Ca^{2+} pump activity may be responsible for an increase in the cytoplasmic Ca^{2+} concentration and it could disrupt the homoeostasis of the cell and cause the toxicity to the kidney [37].

These observed electrolytic changes in *Indigofera aspalathoides* treated fibrosarcoma bearing animals were reversed to normal levels.Ca^{2+} ATPase enzyme, responsible for active Calcium transport, is extremely sensitive to Hydrogen

Peroxide and this may lead to inhibition of enzyme activity. In the present study, Ca^{2+} ATPase activity was found to decrease in fibrosarcoma bearing animals as compared to control animals. The increase in renal endoplasmic reticulum pump activity may be responsible for to increase in the cytosolic Calcium concentration and the activity could disrupt the normal Calcium homoeostasis of the cell and causes toxicity to kidney.Aqueous extract of *Indigofera aspalathoides* administration could modify the altered membrane fluidity and thereby improved the cell membrane integrity by modulating the activity of membrane ATPases.

CONCLUSION

The above study clearly indicates that *Indigofera aspalathoides* has a profound carcinoprevention property. Further study should is needed to prove its antineoplastic effects on patients in future.

Acknowledgments
The corresponding author is grateful to Prof. Dr. B. Nagarajan, Dept. of Microbiology and Tumor Biochemistry, Cancer Institute, Chennai, India, for his technical advice and help for production of fibrosarcoma in albino rats.
The project was a part of the Ph.D work of SSK and it was self stipendiary and no funding like grants, stipends etc. were availed.

REFERENCES

[1] Mendelshon J; Howly PM; Israel MA; Liotta LA. *The molecular basis of cancer* (2nd Ed.) **2001**. Heartwell Health Science Company, pp 621-47.
[2] Campbell PN. *Arogya J Health Sci*, 1**984**, X: 1-8.
[3] Palani V; Senthil Kumar RK; Govindasamy S. *J. Ethnopharmacol.*, **1999**, 65: 257-265.
[4] Elekofehinti OO; Kade I J. *Der Pharmacia Lettre*, **2012**, 4 (5):1352-1359
[5] Choudhary GP. *Der Pharmacia Lettre*, **2012**, 4 (2):414-417
[6] Kirtikar KR; Basu BD. *Illustrated Indian Medicinal Plants III*, Revised and Enlarged Edition, **2000**, Vol. 3:997-99.
[7] Rajakapoor B, Murugesh N, Decen Chodon, Sakthisekaran D. Biol. Pharm. Bull., **2005**, 28(2): 364-66.
[8] Selva Kumar S; Karrunakaran CM; Ram Krishna Rao M; Balasubramanian MP. *Asian Pecific J of Cancer Prevention*, **2011**, 11: 1833-37.
[9] Kumar SS; Karrunakaran CM; Rao MRK; Balasubramanian MP. *J Carcinog*, **2011a**, 10:1-8.
[10] Selva Kumar S; Ram Krishna Rao M; Balasubramanian, MP. *Int. J. Can. Res.***2011 b** 7(2): 144-51.
[11] Selva Kumar S; MRK Rao; Balasubramanian MP. *Res J. Medicinal Plants*, **2011c**, (6): 747-55.
[12] Adetuyi FO; Ibrahim TA; Jde-ojei; Ogundahunsi GA. *African Journal of Biotechnology*, **2009**, 8(7):1305- 9.
[13] Trease GE; Evans WC. *Pharmacognosy*, **1989,** 11th edn. Brailliar Tiridal can Macmillian Publishers.
[14] Sofowora A. *Medicinal plants and traditional medicine in West Africa*. **1982**.John Wiley and Sons, New York. pp: 256.
[15] Finar, ll. *Organic chemistry*: **1983**. 25th edition, 696-765, Longman, London.
[16] Nagarajan B; Sankaran S. *Ind. J. Cancer*, **1973**, 10: 83- 4.
[17] Fiske CH; Subbarow YV. *J Biol Chem.*, **1925**, 66: 375-400.
[18] Dodge JT; Mitchell C; Hanahan DJ. *Arch Biochem Biophys*, **1963**, 100: 119-30.
[19] Quist EE. *Biochem. Biophys Res Commun*, **1980**, 92: 631-37.
[20] Hjerton S; Pan H. *Biochem Biophys Acta*, **1983**, 728: 285-88.
[21] Bonting SI. In, *Membranes and Ion Transport*. **1970**, (Ed. Bitter, E. E.). Wily Inter Sciences, London. Vol I, 257-363.
[22] Ohnishi T; Suzuki T; Suzuki Y; Ozawa K. *Biochem. Biophys. Acta*., **1962,** 684: 67-74.
[23] Lowry OH; Rosebrough NJ; Farr AL; Randall RJ. *J Biol Chem*, **1951**, 193: 265-75.
[24] Selva Kumar S; Rao MRK; Balasubramanian MP. *ISRN Pharmacology*, **2012**; Doi: 10; 5402/2012/134356.
[25] Swami SB; Thakor NJ; Patil MM; Haldankar PM. *Food and Nutrition Sciences*, **2012** (In Press)
[26] Aggarwal BB; Van Kuiken ME; Iyer LH. *Exp Biol Med*, **2009**, 234: 825-49.
[27] Smith TJ; Hong JY; Wong ZY; Yang CS. *Acad Ann Noi Sci*., **1995**, 768: 85-90.
[28] Fransworth NR;Soegarto DD. In: Akerele, Heywood V. Synge H (eds.). *Conservation of Medicinal plants*. **1991**, Cambridge university press, Cambridge: 25-51.
[29] Drake IIEN; Skypolk HH. *Cancer Res.*, **1989**, 49:4210-15.
[30] Notachin YV; Bakhteeva VT; Karpenko LA. *Ren. Fail.*,**1994,** 16: 687-96.

[31] Courijault GF; Hoet D; Leroy D; Tautain HJ. *J Pharmacol Exp Ther*, **1994**, 270: 1097-1104.
[32] Schilsky RL; Anderson T. *Ann. Int. Med*., **1979**, 90: 929-31.
[33] Mavichak V; Wong NLM; Quamme GA; Divks JH; Sutton RAL. *Kid. Int.*, **1985**, 28: 914-21.
[34] Farber J. *Life sciences*, **1981**, 47:114-23.
[35] Orrenius S; Makonsky DJ; Nicotera P. In: Heilmann(ed.). **1987**, *Ca^{2+} dependent process in the liver*. Lancaster, MTP Press, Boston, pp-181-89.
[36] Prasad SB; Giri A. *Med Sci Res*, **1999**, 27:459-62.
[37] Jones TW; Chopra S; Kaufman JS; Flamenbaum W; Trump BP. *Lab. Invest.*, **1985**, 52:363-74.