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Anti-Carcinogenic Activity of Multi Herbal Powder. An Indigenous Herbal Preparation

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Cancer chemopreventive potential of multi-herbal formulation was studied on induced fibro sarcoma development in swiss albino mice. Oral administration of multi herbal powder was found to reduce the tumour weight in induced mice and increased their life span when compared with the induced untreated animals. Also enzymes such as g-glutamyl transpeptidase, Glutathione-s-transferase, Lipid peroxidase, Glutamate pyruvate transaminase and Alkaline phosphatase levels were decreased from the induced with treated animals when compared with induced untreated animals. The results revealed that the multi herbal powder can be recommended as a herbal health and as anti-cancer agent.

Key words: Anti – Carcinogenic, -glutamyl transpeptidase, Glutathione-s-transferase, Lipid peroxidase, Glutamate pyruvate transaminase, Alkaline phosphatase.

Cancer is a disease characterized by chronically unregulated cell growth (Clark., 1991). Cancer is a biological phenomenon resulting from damage inflicted to the genetic material of different types of cells (Lathia and Blum, 1989; Willet, 1994).

Cancer is the second largest killer disease in the developed countries. It is estimated that cancer accounts for more than 20% of the deaths in United States (Satyanarayana, 2006). It is difficult to eliminate mutagenic, carcinogenic factors present in our environment; but it is possible to diminish the risk of cancer through the use of simple dietary and herbal recommendations (carpenter 1972; Genta et al., 1974; Hayatsu, 1982; Block et al., 1992; Sharma 1990; Sharma et al., 1994; Polasa, 1994).

Cancer chemoprevention is defined as the use of chemicals or dietary components to block, inhibit or reverse the development of cancer in normal or preneoplastic tissue. (Talalay et al., 1995). A large number of potential chemopreventive agents has been found to function by a variety of mechanisms directed at all major stages of carcinogenesis.

Multi herbal powder preparation consisting of *Argemone mexicana, Corallocarpus epigaceus, Cassia angustifolia, Aristolochia indica, Calotropis procera, Curcuma longa, Vitex negundo, Elephantopus scaber, Gloriosa superba* and *Withania somnifera*. These plants are being in indigenous herbal medicine as an anticancer

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activity. The main aim of this study is to investigate the anti-carcinogenic activity of this herbal medicine.

MATERIAL AND METHODS

Animals

The Swiss albino mice (20-25 gm) were obtained from Tetrex Biological supply house, Madurai. They were housed in ventilated cages in 12 hrs light and 12 hrs of darkness. The animals were fed at evening by specially provided wheat flour, carrots, papaya fruits and lemon rice at morning every day and water freely available adlibitum.

Materials

20 - Methylcholanthrene and all of the chemicals were purchased from Ponmani Chem-Glass agencies. (Madurai, Tamilnadu). All other chemicals used were of analytical grade.

Drug preparation

Multi Herbal Powder was dissolved in honey and administrated orally to the animals.

Determination of the effect of multi-herbal powder on 20-methylcholanthrene induced fibrosarcoma.

Swiss albino mice (20-25 gm,30/group) were used for the study. Hair was removed from the dorsal side of all animals and a single dose of 20-methylcholanthrene (200 µg / 0.1 ml of DMSO / Mice) was injected subcutaneously on the dorsal side. The tumours which appear in about 60 days.

The animals were divided into six groups. Group - I is control and not induced. Group - II, which received 20-methylcholanthrene only, untreated and was kept as control. Groups III, IV, V and VI ware induced by 20-methylcholanthrene and treated with different concentrations (100, 200, 300 and 400mg kg^{-1}) of multi herbal powder medicine, respectively. This dosage, which was non toxic to the animals. Administration of the drug started 60 days after 20-methylcholanthrene injection and continued for 90 days, every day orally. The control group-I and induced group-II were kept without any treatment. The animals were sacrificed after every 15th day treatment and serum, liver, Kidney were isolated.

The serum, liver and kidney parameters were estimated to assess the effect of multi herbal powder medicine on carcinogenesis. Serum levels of γ-glutamyl transpeptidase activity were measured by kinetic method using an AUTOPAK GGTP diagnostics kit (Sood,1994) and serum glutamate pyruvate transaminase activity was estimated by Wroblewski and La Due (1956); Henry *et al.* (1960). Alkaline phosphatase activity was estimated in serum (PNPP - method). Glutathione - s - trnasferase activity was estimated in liver (Habig *et al.,* 1974). Lipid peroxidase in kidney was estimated by Devasagayam et al., (2003).

Statistical analysis

Results were expressed as mean SD and evaluated by one-way ANOVA.

RESULTS

Multi herbal powder medicine could inhibit fibrosarcoma development induced by 20-methylcholanthrene in a dose dependent manner. Oral administration of the drug (100, 200 and 300 mg kg^{-1}) was found to reduced tumour weight up to 3.107 0.23, 2.402 0.11 and 2.104 0.01 respectively (Table 1), When compared with induced untreated animals. The 400mg of multi herbal powder was found to disappear of tumour. The life span increased by 180.7, 256.44, 320.6 and 368.41 days respectively (Table 2).

γ-Glutamyl transpeptidase enzyme activity was decreased by 58.6 4.77, 52.4 3.5, 50.4 4.89 and 20.0 4.35 IUL^{-1} respectively in serum (Table 3), and glutathione-s-transferase activity was decreased by 1108 5.87, 941.6 26.44, 851.8 19.01 and 380.2 7.79 n mol / mg protein respectively (Table 4), when compared with the induced untreated animals.

Administration of multi herbal power also decreased the Lipid peroxidase by 0.29 0.02, 0.25 0.04, 0.20 0.03 and 0.21 0.04 n mol / mg protein respectively (Table 5). The Glutamate pyruvate transaminase levels decreased by 28.74 4.99, 20.76 3.55, 21.03 2.61 and 19.46 2.77, IUL^{-1}, respectively (Table 6). The Alkaline phosphatase levels were decreased by 46.2 2.38, 43.6 7.12, 34.6 2.96 and 31.2 3.70 UL-1, respectively (Table 7) when compared with induced untreated animals.

Table 1. Tumour weight of the 20MC induced with herbal powder treated Mice of every 15th day of sacrificed up to 90 days.

	15th Day	30th Day	45th Days	60th Day	75th Day	90th Day
Control	-	-	-	-	-	-
Induced	6.419 ± 0.23	6.448 ± 0.27	6.495 ± 0.33	7.700 ± 0.39	8.360 ± 0.33	9.297 ± 0.21
100 mg Hp	5.773 ± 0.15	5.358 ± 0.11	4.944 ± 0.46	4.003 ± 0.26	3.572 ± 0.44	3.107 ± 0.23
200 mg Hp	5.579 ± 0.18	5.060 ± 0.12	4.518 ± 0.24	3.583 ± 0.34	2.865 ± 0.17	2.402 ± 0.11
300 mg Hp	5.245 ± 0.06	5.115 ± 0.06	4.360 ± 0.19	3.228 ± 0.06	2.558 ± 0.10	2.104 ± 0.01
400 mg Hp	5.080 ± 0.03	3.062 ± 0.04	1.854 ± 0.38	-	-	-

Values are mean ± SD; N = 5

Table 2. Increase in life span of mice with 20 MC induced (fibrosarcoma) upon the treatment with herbal powder as anticancer agent without sacrifice

20.10.07	Number of days surviving animals						Mean±SD	ILS
	Mice I	Mice II	Mice III	Mice IV	Mice V	Mice VI		
Control	-	-	-	-	-	-	-	-
Induced	28	39	45	57	65	73	51.2±16.88	
100 mg Hp	112	126	430	148	161	185	143.7±26.56	180.7
200 mg Hp	133	150	168	189	220	236	182.5±40.14	256.44
300 mg Hp	151	187	196	228	256	274	215.33±45.98	320.6
400 mg Hp	178	192	233	264	280	292	239.83±47.06	368.41

Values are mean ± SD; N = 5

Table 3. Estimation of γ- glutamyl transcriptase (IU/L) activity in serum of control 20 MC induced and herbal powder treated mice of every 15th day of sacrificed up to 90 days

	15th Day	30th Day	45th Days	60th Day	75th Day	90th Day
Control	20.2±2.58	28.2±2.86	31.8±2.58	33.8±1.92	34.6±3.50	35.2±3.11
Induced	70.0±7.03	102.2±8.13	136.2±4.20	145.2±3.96	153.4±7.12	156.0±4.84
100 mg Hp	141.8±5.14	123.6±6.10	97.0±6.32	85.4±2.70	68.6±2.88	52.4±3.5
200 mg Hp	120.8±6.37	93.6±3.36	88.3±6.05	75.6±3.64	60.2±3.11	58.6±4.77
300 mg Hp	71.4±2.30	68.2±3.03	63.0±2.54	59.8±4.54	56.8±4.02	50.4±4.89
400 mg Hp	53.4±2.07	41.2±2.58	34.0±2.73`	27.2±4.14	22.4±2.07	20.0±4.35

Values are mean SD; N = 5 Normal vmalues = 8.0-37.0 IU/L (30°C)

Table 4. Estimation of Bio transformation enzyme Glutathiore - S - transferase (nmoleMin-1 (Mg protein) in liver of control, 20MC induces and all treated mices of every 15th days sacrificed, upto 90 days

	15th Day	30th Day	45th Days	60th Day	75th Day	90th Day
Control	579.8±12.69	539.4±11.08	511.2±6.76	476.2±16.58	420.8±7.46	377.2±26.52
Induced	1177.6±35.92	1369.4±20.77	1446.6±40.26	1526.6±12.65	1634.0±15.04	1859.2±44.53
100 mg Hp	1288.8±20.32	1246.4±6.91	1208.8±9.88	1170.4±22.47	1144.4±12.34	1108.0±5.87
200 mg Hp	1203.8±10.15	1173.4±13.04	1107.2±8.07	1057.0±22.64	960.4±27.79	941.6±26.44
300 mg Hp	1137.8±29.75	1014.8±7.15	912.4±9.76	894.0±7.07	879.2±11.73	851.8±19.01
400 mg Hp	1012.4±6.02	917.8±11.25	756.2±22.32	460.6±28.43	384.4±10.78	380.2±7.79

Values are mean SD; n=5

Table 5. Estimation of Lipid peroxidase (nmol/mg protein) in kidney of control 20 MC induced and herbal powder treated mice of every 15th day of sacrificed upto 90 days

	15th Day	30th Day	45th Days	60th Day	75th Day	90th Day
Control	0.26 ± 0.02	0.29 ± 0.01	0.32 ± 0.02	0.34± 0.04	0.36 ± 0.02	0.37 ± 0.02
Induced	0.33 ± 0.04	0.52 ± 0.02	0.65 ± 0.05	0.75 ± 0.04	0.80 ± 0.04	0.87 ± 0.03
100 mg Hp	0.80± 0.01	0.73 ± 0.02	0.61 ± 0.03	0.44 ±0.04	0.38 ± 0.02	0.29 ± 0.02
200 mg Hp	0.62 ± 0.02	0.59 ± 0.03	0.54 ± 0.02	0.39 ± 0.03	0.34 ± 0.04	0.25± 0.04
300 mg Hp	0.48 ± 0.02	0.41 ± 0.06	0.37 ± 0.02	0.29 ± 0.01	0.25 ± 0.03	0.20 ± 0.03
400 mg Hp	0.34 ± 0.04	0.33 ± 0.01	0.30 ± 0.01	0.27 ±0.03	0.23 ± 0.02	0.21± 0.04

Values are mean SD; N = 5

Table 6. Estimation of serum glutamate pyruvate transaminase (IU/L) of control 20 MC induced and herbal powder treated mice of every 15th day of sacrificed upto 90 days

	15th Day	30th Day	45th Days	60th Day	75th Day	90th Day
Control	23.5 ± 1.54	25.71 ± 3.22	31.2 ± 6.51	36.38 ± 1.42	38.72 ± 2.56	40.39 ± 3.29
Induced	29.2 ± 2.39	41.68 ±16.69	67.06 ± 4.59	79.85 ± 2.23	86.78 ±4.40	90.98 ± 3.58
100 mg Hp	81.02 ± 3.54	71.57 ± 5.88	58.85 ± 2.80	40.72 ± 1.75	32.45 ± 1.55	28.74 ± 4.99
200 mg Hp	70.12 ± 9.16	65.83 ± 7.98	62.20 ± 3.72	48.72 ± 2.21	35.85 ± 3.84	20.76 ± 3.55
300 mg Hp	48.90 ± 6.73	44.38 ± 5.44	40.66 ± 6.27	35.06 ± 3.73	31.71 ± 2.06	21.03 ± 2.61
400 mg Hp	27.63 ± 4.99	24.94 ± 2.66	22.74 ± 1.76	18.35 ± 2.97	20.24 ± 2.88	19.46 ± 2.77

Values are mean SD; N = 5 Normal values = 5.0-40.0 IU/L (37°C)

Table 7. Estimation of serum Alkaline Phosphatase (U/L) activity of control, 20 MC induces and induces with herbal powder treated mices of every 15th days sacrificed, upto 90 days.

	15th Day	30th Day	45th Days	60th Day	75th Day	90th Day
Control	31.2 ± 2.58	42.8 ± 2.38	46.8 ± 2.86	67.6 ± 6.06	90.2 ± 4.43	98.8 ± 2.56
Induced	49.8 ± 5.21	75.2 ± 7.79	109.6 ± 5.17	125.2 ± 3.96	147.2 ± 5.11	160.0 ± 3.80
100 mg Hp	148.4 ± 4.39	128.8 ± 7.59	115.8 ± 6.87	76.8 ± 5.76	60.0 ±4.06	46.2 ± 2.38
200 mg Hp	125.2 ± 4.54	117.8 ± 6.94	96.8± 5.97	73.4 ± 4.50	57.4 ± 4.61	43.6 ± 7.12
300 mg Hp	96.6 ±5.31	87.6 ± 4.44	83.2 ± 5.63	65.0 ± 4	49.4 ± 3.04	34.6 ± 2.96
400 mg Hp	64.2 ± 4.76	57.6 ± 3.64	52.2 ± 2.86	48.2 ± 5.49	43.6 ± 3.04	31.2 ± 3.70

Values are mean SD; N = 5 Normal values = 20-100 U/L

DISCUSSION

Herbal preparations are being used in alleviating several diseases. Countries such as India and China have large number of traditional medicines which have not yet bean explored scientifically. Lycovin – an indigenous herbal medicines are prescribed not only to reduce suffering but also to prevent disease produced by pathophysiological changes. Even though cancer is one of the most difficult diseases to be treated, its prevention could be achieved by (a) avoidance of cancer-inducing substances; (b) chemo-preventive agents that can inhibit the metabolism of carcinogen or cause its detoxification (Williams, 1971), (c) immunostimulators which can destroy cancer cells by augmenting the immune responses; (d) inhibition of signal transduction pathway which can either inhibit the conversion of normal cells the cancer cells, reduce

its growth capability and destroy cells by increasing the recognition by the immunocompetent cells (Patterson & Subar., 1992).

Several types of compounds numbering more then 2000 chemicals among which may of them are from plant origin, have been reported to inhibit chemically induced carcinogenesis (Soudamini and Kuttan, 1989; Unnikrishnan and Kuttan, 1990).

The compaining of three plants of *W.somnifera, C.longa and P.cuspidatum* together, the growth inhibitory effects could be several – fold enhanced than as a single agent (Bonham et al., 2002). Lower concentrations showed that the *W.Somnifera*, *C.longa* and *P.cuspidatum* are most effective against different prostate cancer cells lines of varying metastatic potential and showed only a moderate effect on BG – 9 normal skin fibroblasts (Rao, et al., 2004). Curcumin acts as potent anti-carcinogenic compound. It induces apoptosis and inhibits cell cycle progression, both of which are instrumental in preventing cancerous cell growth in rat aortic smooth muscle cells (Chen, et al., 1998).

Bone marrow cells from Brahma Rasayana (BR), Aswagandha Rasayana (AR) treated animals also showed an enhanced proliferation in vitro. Recently, both BR and AR could protect the mice from cyclophosphamide radiation induced myelo suppression (Praveen Kumar et al., 1994-1999).

The results of this study indicated that an anti-carcinogenic activity of multi-herbal powder on Swiss albino mice. This multi-herbal powder treatment was found to reduce the g - Glutamyl transpeptidase, Glutamate pyruvate transaminase and Alkaline phosphatase levels in serum, and also found to reduce the Glutathione - s - transferase and liver and Lipid peroxidase in kidney. Multi herbal power treatment reduced the tumour weight and increased the life span compared with the induced untreated mice.

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