Research Article

Antioxidant Potential of the Roots of *Coleus forskohlii* in Balb/C Mice with DLA Tumor

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ABSTRACT

A study has been carried out to evaluate the antioxidant status of the roots of *Coleus forskohlii* against DLA tumor bearing mice. The enzymic antioxidants like catalase, superoxide dismutase, glutathione S-transferase, glutathione peroxidase and glutathione reductase and non enzymic antioxidants such as glutathione and ascorbic acid were assessed. The antioxidant analysis in the mice revealed that the enzymic antioxidants such as catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase activities were found to have decreased in the DLA treated mice compared to the untreated control. The oral administration of the root extracts of *Coleus forskohlii* has significantly increased the enzymic antioxidants. Among the extracts, the methanolic extract showed the maximum enzyme activities. The non enzymic antioxidants level such as glutathione and ascorbic acid were found to be decreased in the mice with DLA tumor compared to the untreated control. The levels were raised significantly in the mice, treated with the methanolic extract of the *Coleus forskohlii* roots.

Keywords: *Coleus forskohlii*, DLA tumor cells, enzymic antioxidants, Non-enzymic antioxidants, reactive oxygen species

INTRODUCTION

The highly reactive free radicals and reactive oxygen species (ROS) that are present in the biological systems from a wide variety of sources may oxidize proteins, lipids or DNA and can initiate degenerative disease[1]. To protect against the toxic effects of ROS and to modulate the physiological effects of ROS, the cell has developed an intricately regulated antioxidant defense system. Oxidative stress occurs in a cellular system when the production of reactive oxygen species (ROS) exceeds the antioxidant capacity of the system. Oxidative stress plays an important contributory role in the process of aging and pathogenesis of numerous diseases like cancer. Improved antioxidant status helps to minimize the oxidative damage and thus delays or decreases the risk for developing many chronic age related, free radical induced diseases[2].

The body possess defence mechanisms against free radical-induced oxidative stress, which involve preventative mechanisms, repair mechanisms, physical defences and antioxidant defences. Enzymic antioxidant defences include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidise (GPx), glutathione S-transferase (GST), glutathione reductase (GR) and the nonenzymic antioxidants are ascorbic acid (vitamin C), glutathione (GSH), carotenoids, flavonoids, etc. All these act by one or more of the mechanisms like reducing activity, free radical-scavenging potential, complexing of pro-oxidant metals and quenching of singlet oxygen[3]. The generation and the subsequent involvement of free radicals in cancer have prompted to study the antioxidant potential of *Coleus forskohlii*. In order to consider a plant extract as an effective antioxidant, it should act as such under both *in vivo* and *in vitro* conditions and it should render lymphocytes more resistant to oxidative challenges. Therefore, the present study has been designed to explore the effect of different extracts of the root of *Coleus forskohlii* on plasma antioxidant status in *Balb/C* mice with DLA tumour.

MATERIALS AND METHODS

The plant material *Coleus forskohlii* was collected from Tamil Nadu Agricultural University, Coimbatore and was duly authenticated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Southern Regional Centre, Tamil Nadu Agricultural University, Coimbatore. The roots of the plants were cut into pieces and dried under shade for a week. The shade-dried roots were coarsely powdered and weighed. Extracted each 100 g of the powder in 500 ml each of 70% petroleum ether, chloroform, acetone and methanol respectively using soxhlet apparatus. The extracts were concentrated to dryness in a rotary evaporator under reduced pressure and controlled temperature (40-50°C). The crude extracts yielded a dark brown solid, weighing approximately 40g. The extracts were preserved in a refrigerator at 4°C for further use.

Healthy male Balb/C mice of approximately with the same weight (25-30 grams) were procured from N.G.P. College of Pharmacy, Coimbatore. The mice were fed with normal laboratory diet and water *ad libitum* and acclimatized for a week under laboratory conditions. The study protocol was approved by the IAEC. The mice were divided into twelve groups of 6 each to determine the enzymic and nonenzymic antioxidants of the different extracts of the roots of *Coleus forskohlii*.

The extraction was filtered and the solvent was removed by distillation under reduced pressure. A brown coloured

fumy residue was obtained. It was then dissolved in 0.3% carboxy methyl cellulose and was used for the study. The mice were given oral dose of extracts after the 1st day of induction of cancer. All the treatments were given 24 hours after the tumour inoculation, daily once for 21 days. After the last dose and 24 hours fasting, the mice were killed for the study of biochemical parameter in liver.

Assessment of Enzymic Antioxidants

The superoxide dismutase was assayed by the method of Kakkar.[4] The catalase activity was assayed by the method of Sinha[5]. The method of Rotruck[6] was used for the assay of glutathione peroxidase. Glutathione-S-transferase was assayed by the method of Habig.[7] Glutathione Reductase was assayed by the method of Goldberg and Spooner[8].

Assessment of Non enzymic Antioxidants

The glutathione content was determined by the method of Moron.[9] The method of Omaye[10] was followed for the determination of ascorbic acid.

Statistical Analysis

One-way Analysis of Variance (ANOVA) was used to determine the statistical significance. 'P' value of 0.05 or less was considered as significant.

RESULTS AND DISCUSSION

Superoxide Dismutase (SOD) and Catalase

Table 1: Activities of Superoxide Dismutase and Catalase in the Liver of Mice

Groups	Treatment		Superoxide dismutase[#]	Catalase [$]
1	Untreated Control	UC	4.42 ± 0.28[a]	50.56 ± 1.88[a]
2	Vehicle Control	VC	4.39 ± 0.19[a]	49.43 ± 0.10[a]
3	Petroleum ether	CPE	4.29 ± 0.02[a]	48.76 ± 0.03[a]
4	Chloroform	CCF	4.04 ± 1.50[a]	49.75 ± 0.05[a]
5	Acetone	CA	3.18 ± 0.20[a]	50.00 ± 0.90[a]
6	Methanol	CM	4.40 ± 0.34[a]	47.45 ± 0.75[a]
7	DLA Control	DC	2.10 ± 0.20[e]	20.87 ± 1.13[c]
8	Petroleum Ether extract	DPE	2.45 ± 0.04[c]	21.99 ± 1.65[c]
9	Chloroform extract	DCF	2.29 ± 0.02[d]	22.83 ± 1.76[c]
10	Acetone extract	DA	2.19 ± 0.02[e]	21.44 ± 0.57[c]
11	Methanol extract	DM	3.96 ± 0.12[b]	48.46 ± 2.86[a]
12	Methotrexate	DMT	4.5 ± 0.20[a]	42.00 ± 1.21[b]
	CD (5%)		0.19	2.20

Values are mean ± SD of six mice

Means followed by common superscripts do not differ significantly at 5% level

[#] Values are expressed for 50% inhibition of Nitroblue tetrazolium /min/mg protein

[$] Values are expressed in µ moles of H_2O_2 consumed/min/mg protein

Table 1 gives the effect of different extracts of the roots of *Coleus forskohlii* on the activities of superoxide dismutase and catalase in the liver of mice induced with DLA cells. The activity of SOD was significantly decreased in DLA bearing mice when compared to the untreated control mice. Among the different extracts of *Coleus forskohlii,* the methanolic extract showed significant increase (P<0.05) in SOD activity compared to chloroform, acetone and petroleum ether extracts. SOD is a ubiquitous chain breaking antioxidant found in all aerobic organisms. It is a metalloenzyme, widely distributed in all cells and plays an important protective role against ROS induced oxidative damage. It has been demonstrated both *in vivo* and *in vitro* that the antioxidant enzyme activities are altered in cancer. The manganese superoxide dismutase (MnSOD), a mitochondrial antioxidant enzyme, is lowered in most types of primary cancers and cancer cell lines[11].

The catalase activity was decreased significantly (P<0.05) in DLA control mice when compared with the untreated control mice. The treatment with methanolic extract brought back the catalase activity to normal level compared to the other extracts which was also found to be higher than the standard drug. Both SOD and catalase play an important role in the elimination of ROS derived from the redox process of xenobiotics in liver tissue.

It has been suggested that catalase and SOD are easily inactivated by lipid peroxides and ROS. It has been reported that DLA bearing mice showed decreased activity of SOD in the liver and this might be due to loss of Mn^{++} SOD[12].

Tumor cells have increased the rate of metabolism compared to normal cells which would typically lead to increased number of reactive oxygen species.

The reduced activities of both the enzymes in the cell might be due to abnormalities in the regulation of SOD and CAT genes in the pluripotent stem cells.

Alternatively, it could be due to post translational modification of the enzymes by free radicals[13].

Glutathione Peroxidases (GPx)

The effect of different extracts of the roots of *Coleus forskohlii* on the activities of glutathione peroxidase, glutathione S- transferase and glutathione reductase in DLA induced mice is shown in Table 2.

Glutathione peroxidase activity was reduced in control DLA mice when compared to the untreated control mice.

The petroleum ether, chloroform and acetone extracts treated mice recorded a non significant GPx activity when compared to the untreated control mice.

Whereas the methanolic extract treated mice showed a significant (P<0.05) increase in GPx activity similar to the methotrexate standard treated mice. GPx catalyses the reduction of H_2O_2 at the expense of reduced GSH thereby protecting mammalian cells against oxidative damage[14].

The decreased activity in the present study might be due to the less availability of the substrate GSH. GSH could directly scavenge and eliminate the toxicity, thereby minimizing the toxic effects which definitely indicate the antioxidant potency of the plant extract.

Table 2: Activities of Glutathione Peroxidase, Glutathione S-Transferase and Glutathione Reductase

Groups	Treatment		Glutathione Peroxidase#	Glutathione S-Transferase$	Glutathione Reductase$
1	Untreated Control	UC	7.26 ± 0.19[a]	41.74 ± 0.50[d]	35.64 ± 0.21[a]
2	Vehicle Control	VC	7.14 ± 0.09[a]	41.50 ± 0.70[d]	33.63 ± 2.18[a]
3	Petroleum ether	CPE	7.21 ± 0.02[a]	40.89 ± 0.06[d]	34.17 ± 1.36[a]
4	Chloroform	CCF	7.13 ± 0.05[a]	42.35 ±1.05[d]	34.33 ± 1.32[a]
5	Acetone	CA	7.24 ± 0.01[a]	41.4 7± 0.05[d]	34.50 ± 0.41[a]
6	Methanol	CM	6.95 ± 0.21[a]	42.35 ± 2.00[d]	35.60 ± 1.10[a]
7	DLA Control	DC	4.26 ± 0.15[f]	73.44 ± 3.03[a]	20.63 ± 2.18[d]
8	Petroleum Ether extract	DPE	4.42 ± 0.13[e]	71.40 ± 1.03[a]	33.05 ± 1.36[b]
9	Chloroform extract	DCF	4.71 ± 0.17[d]	69.52 ± 2.22[b]	25.12 ± 0.56[c]
10	Acetone extract	DA	4.40 ± 0.45[e]	70.38 ± 1.27[b]	26.08 ± 0.72[c]
11	Methanol extract	DM	6.53 ± 0.16[c]	45.36 ± 1.28[c]	34.67 ± 0.52[a]
12	Methotrexate	DMT	6.95 ± 0.21[b]	47.56 ± 2.85[c]	35.90 ± 1.51[a]
	CD (5%)		0.20	2.51	1.53

Values are mean ± SD of six mice
Means followed by common superscripts do not differ significantly at 5% level.
# µg of glutathione /min/mg of protein
$ n moles of CDNB complexed/min/mg/protein

Glutathione-S-Transferase (GST)

GST was increased in the DLA control mice significantly (P<0.05) compared to the untreated control mice.

The petroleum ether, chloroform and acetone extracts treated mice recorded a non significant GST activity, compared to the control mice (P<0.05).

The methanolic extract treated mice showed a significant decrease (P<0.05) in GST activity which was comparable with the standard, methotrexate treated mice.

The methanolic extract could activate GPx and GST activities. Alteration in circulating oxidants and free radical scavengers like GST has been involved in the treatment of various malignancies. An increase in GST in oral tumor tissue has also been reported by Subapriya[15].

GST also plays a critical role in detoxification mechanism that functions primarily in conjugating functionalized P_{450} metabolites with endogenous ligand (GSH) favouring their elimination from the organism.

There are convincing evidences to support induction of GST and protection against a wide spectrum of cytotoxic, mutagenic and carcinogenic agents[16].

Glutathione Reductase (GR)

GR activity was significantly (P<0.05) reduced in the DLA control mice compared to the untreated control mice.

The methanol extract treated mice showed a significant (P<0.05) increase in GR activity similar to the standard methotrexate which was followed by petroleum ether extract.

Non Enzymic Antioxidants

Non enzymatic antioxidants play a crucial role in scavenging or disposing lipid peroxidation byproducts when they are excessively generated in the body. Tumor tissues sequester nutrients and antioxidants from circulation to combat the deleterious effects of reactive oxygen species and for their abnormal growth. Ascorbic acid, the most important antioxidant in the plasma, scavenges a variety of free radicals. Reduced glutathione is the most powerful intracellular antioxidant and the molar ratio of reduced glutathione to oxidized glutathione serves as an important marker of the antioxidative capacity of the cell[17]. The effect of administration of the root extracts of *Coleus forskohlii* on non enzymic antioxidants glutathione and ascorbic acid in the control and the DLA induced mice was depicted in Table 3.

Table 3: Levels of Glutathione and Ascorbic Acid in the Mice Liver

Groups	Treatment		GSH (mg/g tissue)	Ascorbic acid (mg/100g tissue)
1	Untreated control	UC	4.47 ± 0.03[a]	99.61 ± 4.05[a]
2	Vehicle Control	VC	4.53 ± 0.02[a]	98.42 ± 3.35[a]
3	Petroleum ether	CPE	4.41 ± 0.05[a]	99.01 ± 5.41[a]
4	Chloroform	CCF	4.49 ± 0.01[a]	100.00 ± 3.98[a]
5	Acetone	CA	4.51 ± 0.05[a]	101.41 ± 8.45[a]
6	Methanol	CM	4.40 ± 0.04[a]	97.79 ± 1.55[a]
7	DLA Control	DC	0.7 ± 0.04[e]	64.71 ± 3.40[b]
8	Petroleum Ether extract	DPE	1.43 ± 0.01[d]	68.22 ± 2.02[b]
9	Chloroform extract	DCF	1.92 ±0.02[c]	66.52 ± 1.47[b]
10	Acetone extract	DA	1.22 ± 0.01[d]	65.98 ± 2.11[b]
11	Methanol extract	DM	3.75 ± 0.09[b]	97.51 ± 2.56[a]
12	Methotrexate	DMT	4.26 ± 0.02[a]	96.34 ± 3.72[a]
	CD (5%)		0.28	3.72

Values are mean ± SD of six mice
Means followed by common superscripts do not differ significantly at 5% level.

Glutathione

The GSH content in the liver tissues of normal mice was found to be 4.47 mg/g tissue. Inoculation of DLA drastically decreased the GSH content to 0.7mg/g tissue. Among the different extracts of *Coleus forskohlii*, only methanolic extract had increased the GSH to near normal, whereas the petroleum ether, chloroform and acetone extracts did not show a significant increase which indicated that the methanolic extract of *Coleus forskohlii* root has pronounced effect on GSH levels compared to other extracts.

Glutathione an endogenous intracellular thiol containing tripeptide is an important cellular antioxidant and has been the focus of interest in cancer chemotherapy. The active role of GSH against cellular lipid peroxidation has been well recognized. GSH can act either to detoxify or activate oxygen species such as H_2O_2 or reduce lipid peroxides. It is also involved in many cellular functions

i.e., bioreductive reactions, maintenance of enzyme activity, amino acid transport, protection against oxidative stress, radiation and chemotherapy, detoxification of xenobiotics and drug metabolism. GSH also controls the onset of tumor cell proliferation by regulating protein kinase C[18].

Ascorbic Acid

As seen in Table 3, the level of ascorbic acid, shows a significant ($P<0.05$) decrease in the DLA control mice compared to the level in the untreated control mice. Among the extracts, the methanolic extract caused significant ($P<0.05$) increase in ascorbic acid which is on par with the standard drug, methotrexate. The other extracts did not improve the ascorbic acid content to a greater extent. Decreased levels of vitamin E, vitamin C and GSH were reported in both the human and the experimental carcinogenesis. Lowered levels of vitamin C and reduced glutathione in plasma and erythrocytes were probably due to their utilization by malignant tumor[19]. The decreased ascorbic acid in the DLA induced animals might be due to excessive utilization of these antioxidants for quenching the enormous amounts of free radicals produced. The antioxidants react cooperatively *in vivo* so as to provide greater protection to the organs against radical damage that could not be provided by any single antioxidant. The decreased levels of GSH and vitamin C in the DLA injected animals in the present study indicated the increased rate of lipid peroxidation with a concomitant decrease in the activities of SOD, CAT and GPx. The treatment with *Coleus forskohlii* extract might have effectively controlled the loss of GSH and Vitamin C, thereby maintaining the activities of SOD, CAT and GPx.

CONCLUSION

The *in vivo* antioxidant analysis in the mice revealed that the enzymic antioxidants such as catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase activities were found to have decreased in the DLA treated mice compared to the untreated control. The oral administration of the root extracts of *Coleus forskohlii* has significantly increased the enzymic antioxidants. Among the extracts, the methanolic extract showed the maximum enzyme activities. The non enzymic antioxidants level such as glutathione and vitamin C was found to be decreased in the mice with DLA tumor compared to the untreated control. The levels were raised significantly in the mice, treated with the methanol extract. The administration of methanolic extract of *Coleus forskohlii* has exhibited significant antioxidant activity. Thus the study confirms the protective effects of the *Coleus forskohlii* roots against DLA tumor.

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