PROTECTIVE EFFECT OF *BACOPA MONNIERA* L. ON CYTARABINE INDUCED BIOCHEMICAL CHANGES IN CHICK EMBRYO

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ABSTRACT

Bacopa monniera, a medicinal plant distributed throughout India. Cytosine arabinoside (1- β -arabinofuranosylcytosine; Ara-C) is the most important antimetabolite chemotherapeutic drug used for acute leukemia. In this study we examined the chemoprotective property of an ethanolic extract of *Bacopa monniera* on biochemical changes in chick embryo. CA caused biochemical changes in a concentration and time dependent manner in amniotic fluid, liver and heart tissues. Ethanolic extract of BM given to chick embryo at doses of 2, 4, 6 mg per egg. There is significant decrease in biochemical levels of glucose, protein, urea, uric acid, creatinine and inorganic phosphorus. Enzymatic activities of alkaline phosphatase, lactate dehydrogenase, serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase and malatedehydrogenase were also decreased with dose dependent manner in amniotic fluid, liver and heart tissues.

KEY WORDS

Bacopa monniera, Ara-C, Amniotic fluid, Biochemical parameters and Chick embryo.

INTRODUCTION

In recent years, there is an increasing awareness that certain naturally occurring compounds in plants have protective effects against environmental mutagens, carcinogens and endogenous mutagens (1-4). Dietary intake of such chemopreventive compounds has been suggested as an effective strategy for minimizing the deleterious effects of genotoxins/cytotoxins (5-7).

The drug toxicity is of great concern during pregnancy. In this study we observed the amniotic fluid as an indicator for drug toxicity in developing fetus of chick as model. The use of chick model for toxicological and pharmacological studies is promoted, as the mother does not influence the pharmacokinetics of the drug (8-9).

Bacopa monniera (Scrophulariaceae), commonly known as Brahmi, is a medicinal herb, has been used in folklore medicine and the ancient traditional system of Ayurveda as a nerve

tonic for improvement of intelligence, memory and revitalization of sense organs, to treat epilepsy, insomnia, asthma, rheumatism and also as a diuretic and cardiotonic (10-11). The active constituents of *Bacopa monniera* (BM) are saponins, alkaloids, and phytosterols (12-13). In pharmacological studies, alcoholic extract of BM has been shown to possess anticancer activity against Walker carcinosarcoma 256 in rats (14), growth inhibitory effects on sarcoma 180 cultures (15).

Cytarabine also known as cytosine arabinoside (CA) is one of the most effective anticancer drug used in the treatment of cancer (16-17). The mechanism of action of CA is phosphorylated in the cell, its active metabolite, 1- β -arabinofuranosylcytosine 5'-triphosphate, which inhibits DNA polymerase in competition with deoxycytidine 5'-triphosphate (d-CTP) (18-19). dCTP is an essential component of DNA and induced dCTP deficiency prevents DNA synthesis and cell division. The cytotoxicity of ara-CTP is attributed to inhibit DNA synthesis, and it is therefore, specific to the S-phase in growing cell (20).

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MATERIALS AND METHODS

Preparation of plant extract : The whole plant of *B. monniera*

was collected from Tirumala hills, Tirupati, Chittoor District, Andhra Pradesh. Shade dried and finely powdered plant material was soaked in 95% ethanol by stirring continuously and extracted in the cold after soaking for 48 hrs. The extract was filtered and distilled on to a water bath to a syrupy mass and the last traces of the solvent were removed in vacuum.

Chemicals : Ara-C was purchased from Dabur India Ltd., Pharmaceutical Division, Solan Dist., Himachal Pradesh, India. Freshly laid Bobcock strain fertilized eggs of zero days' old were obtained from Acharya N.G. Ranga Agricultural University (Poultry Science Department), Tirupati, Chittoor Dist., Andhra Pradesh. They were cleaned with wet cotton and placed in sterile egg incubator maintained at 37°C with 65% relative humidity, by keeping a tray full of water. Eggs were rotated manually once in a day and were examined through the candler every alternate day for proper growth and viability. The Ethanolic extract of *Bacopa monniera* was injected in three different doses 2, 4 and 6 mg per egg on the 12th day. The eggs were intoxicated with a single dose of CA on the 15th day of experiment. The embryos were coded into three groups.

- Group I – Control – saline treated
 Group II – embryos received a single dose of cytarabine on 15th day of the experiment.
 Group III – Embryos were administered the alcoholic extract of BM on 12th day and were intoxicated with a single dose of CA on the 15th day of experiment (i.e. 16th day).

The embryos were exposed after 24h of CA administration, the AF and tissues of liver, heart and brain were collected for estimation of various biochemical parameters.

Amniotic fluid analysis : Amniotic fluid (AF) was centrifuged at 3000 g for 10 min and then supernatant was taken for biochemical analysis.

Tissue extract analysis : 10% homogenate of the embryonic liver, brain and heart was prepared by grinding in Tris HCl buffer, pH 7.4. The homogenate was centrifuged at 3000 rpm in Remi refrigerated centrifuged and the supernatant was used for the measurement of biochemical parameters. The protein content was estimated by Bradford method (21), ascorbic acid by Omaye et al., (22) glucose by O-toluidine method (23),

Table 1 : Biochemical components of Amniotic fluid in chick embryo

S. No.	Biochemical Parameter	Control	CA (3 mg)	CA + BM [@]		
				2 mg [@]	4 mg [@]	6 mg [@]
1	Glucose (mg/dl)	54.5±1.6	62.3±0.1*	60.8±0.4*	57.9±0.6*	51.6±1.3**
2	Protein (mg/dl)	16.2±0.1	22.7±0.4*	20.05±0.1*	18.2±0.4*	15.3±0.1*
3	Uric acid (mg/dl)	8.5±0.08	14.3±0.3*	12.6±0.4*	10.1±0.1*	7.6±0.1*
4	Urea (mg/dl)	8.5±0.2	16.9±0.3*	15.2±0.1*	11.8±0.5*	7.35±3.0*
5	Creatinine (mg/dl)	0.3±0.1	0.9±0.02*	0.5±0.05*	0.37±0.02*	0.24±0.01*
6	Sodium (mEq/L)	133.2±2.2	123.0±1.4*	125.1±0.7*	129.4±0.8*	130.3±0.1**
7	Potassium (mEq/L)	2.6±0.4	3.8±0.4*	3.4±0.1*	2.9±0.08	2.07±0.08**
8	Inorganic Phosphorus (mg/dl)	0.3±0.05	0.8±0.05*	0.55±0.05*	0.52±0.01*	0.2±0.01*
9	Ascorbic acid (mg/dl)	3.0±0.1	0.4±0.1*	0.9±0.08*	1.7±0.1*	2.05±0.05*
10	Cholesterol (mg/dl)	37.7±1	20.2±0.8*	25.0±0.4*	32.1±1.3*	34.9±0.3*
11	Calcium (mg/dl)	10±0.1	4.2±0.2*	6.1±0.4*	8.05±0.05*	8.7±0.2*
12	ALP [#]	4.0±0.1	15.4±0.9*	12.6±0.4*	9.2±0.3*	4.8±0.3**
13	SGOT (µmol/min/mg protein)	12.5±0.5	27.5±1.5*	20.7±0.8*	15.4±0.5*	14.6±0.4
14	SGPT (µmol/min/mg protein)	1.9±0.08	6.1±0.16*	5.1±0.2*	4.1±0.1*	2.9±0.3*
15	LDH [~]	12.5±0.2	16.2±0.2*	15.5±0.2*	13.9±0.2*	12.8±0.09
16	MDH [!]	15.4±0.1	18.8±0.2*	17.7±0.2*	16.8±0.2*	15.9±0.2**

* p<0.001 **p<0.05 # Units : µmol phenol liberated/min/mg protein

[!] Units: nmol NADPH oxidized/min/mg protein ~ µmol pyruvate formed/min/mg protein

inorganic phosphorus by Fiske Subbarow method (24), urea by Natelson et al. (25), creatinine by Broad and Sirota (26), uric acid by Caraway (27), cholesterol by Zlatkis et al. (28), sodium and potassium concentration were measured using Flame photometer (ELICO). Marker enzymes of lactate dehydrogenase (LDH) by King (29) method, malate dehydrogenase (MDH) by Mehler et al (30), alkaline phosphatase by King (31), alanine aminotransferase and aspartate aminotransferase (SGOT & SGPT) by King (32) method.

RESULTS

Administration of CA to chick embryo revealed a significant increase in biochemical parameters in AF, liver and heart tissues (Table 1, 2 and 3). Treatment with Bacopa monniera (BM) afforded a significant protection against CA induction. Table 1 represents the biochemical parameters in chick amniotic fluid in controls and CA (CA+BM) treated embryos. BM was given on 12th day and CA treatment was given on the 15th day of incubation and biochemical analysis was done after 24h of CA intoxication.

Significant increase ($p < 0.001$) in glucose, protein, uric acid, creatinine, inorganic phosphorus levels were observed with CA treatment, whereas with CA+BM treatment a significant decrease ($p < 0.001$) in glucose, protein, urea, uric acid and creatinine, inorganic phosphorus levels were observed. Glucose levels were significantly decreased ($p < 0.05$) with 6 mg BM treatment.

Ascorbic acid levels were significantly decreased ($p < 0.001$) with CA treatment. Whereas CA+BM treatment caused significant ($p < 0.001$) increase in ascorbic acid levels. The calcium levels were significantly decreased ($p < 0.001$) with CA treatment. The cholesterol, sodium, potassium levels were significantly decreased with CA treatment, whereas increased in BM treated embryos.

The enzymatic activity of alkaline phosphatase (ALP) was increased considerably ($p < 0.01$) with CA treatment, whereas significant decrease ($p < 0.01$) in CA+BM treated ones. Serum SGOT and SGPT activities were significantly increased ($p < 0.001$) with CA treatment, whereas significantly decreased ($p < 0.001$) with BM treatment.

Table 2 depicts the treatment with CA elevated biochemical parameters of ALP, SGOT, SGPT, LDH and MDH were observed. The levels of protein, inorganic phosphorus and ascorbic acid were decreased. The altered biochemical features were significantly brought towards normalization by treatment with BM extract (Table 2) in liver.

Table 3 depicts the effect of CA on chick embryonic heart. Significant decrease ($p < 0.001$) in protein, inorganic phosphorus, cholesterol and ascorbic acid was observed. The enzymatic levels of LDH and MDH significantly increased ($p < 0.001$) with CA treatment compared to control. The altered protein, inorganic phosphorus, cholesterol and ascorbic acid levels were observed with BM treated embryos. The enzymatic levels of LDH and MDH levels were also altered with BM treated embryos compared to CA treated ones.

Table 2 : Biochemical components of liver tissue in chick embryo

S. No.	Parameter	Control	CA (3.0 mg)	CA + BM [@]		
				2 mg [@]	4 mg [@]	6 mg [@]
1	Protein (mg/100mg dry weight)	18.3±0.1	15.2±0.1*	15.7±0.4*	16.4±0.2*	17.02±0.1*
2	Inorganic Phosphorus (mg/100mg dry weight)	1.8±0.01	0.2±0.04*	0.37±0.04*	0.92±0.08*	1.05±0.1*
3	Ascorbic acid (µg/100mg dry weight)	134.0±1.4	94.0±1.4*	103.8±0.8*	120.5±0.4*	128.4±0.4*
4	ALP #	8.1±0.1	13.2±0.1*	12.1±0.1*	9.8±0.3*	9.07±0.08*
5	SGOT# #	9.8±0.3	18.4±0.1*	14.9±0.2*	12.3±0.1*	10.5±0.2**
6	SGPT# #	6.2±0.08	9.4±0.1*	8.5±0.1*	7.2±0.3*	6.7±0.1*
7	LDH~	72.6±0.1	86.4±0.2*	80.5±0.1*	78.7±0.4*	74.5±1.05**
8	MDH [!]	84.4±0.1	97.2±1.3*	90.2±0.1*	88.1±0.1*	87.3±0.3*

* $p < 0.001$ ** $p < 0.05$ # Units : µmol phenol liberated/min/mg protein

Units : µmol pyruvate liberated/min/mg protein ! Units: nmol NADPH oxidized/min/mg protein ~ µmol pyruvate formed/min/mg protein

Table 3 : Biochemical components of Heart tissue in chick embryo

S. No.	Parameter	Control	CA (3.0 mg)	CA + BM [@]		
				2 mg [@]	4 mg [@]	6 mg [@]
1	Protein (mg/100mg dry weight)	28.3±0.08	19.5±0.1*	20.3±0.1*	24.3±0.1*	26.05±0.8*
2	Inorganic phosphorus (mg/100mg dry weight)	0.9±0.08	0.8±0.08*	0.82±0.1*	0.85±0.08*	0.88±0.1*
3	Cholesterol (mg/100mg dry weight)	24.5±2.1	14.0±1.4*	15.9±0.3*	19.4±0.3*	21.3±0.8**
4	Ascorbic acid	117.0±2.2	92.2±1.0*	98.3±0.1*	101.8±0.3*	112.4±1.3**
5	SGOT# #	4.4±0.1	6.1±0.3*	5.2±0.07*	5.0±0.07*	4.7±0.1**
6	SGPT# #	5.5±0.2	7.5±0.2*	6.5±0.2*	6.2±0.4**	5.5±0.2
7	LDH~	20.4±0.1	26.4±0.2*	24.4±0.2*	23.3±0.1*	22.2±0.4*
8	MDH [!]	36.5±0.2	42.4±0.1*	40.3±0.1*	38.2±0.3*	37.8±0.1*

* p<0.001 **p<0.05 # # Units: µmol pyruvate liberated/min/mg protein

[!] Units: nmol NADPH oxidized/min/mg protein ~ µmol pyruvate formed/min/mg protein

DISCUSSION

In the present study the administration of CA caused significant biochemical changes in AF, liver and heart tissues. The biochemical changes in AF, liver and heart would be well used to extrapolate the findings similar to the CA induced changes in higher model system and even with humans, provided the mechanism by which the damage induced is clearly understood. The levels of glucose was significantly increased with CA treatment in AF compared to controls. The change in glucose level is an indication of alterations on carbohydrate metabolism. It is also major source of energy for the nervous system and erythrocytes. The reversal of increased glucose returned to normal by BM supplementation. Elevated levels of urea, uric acid and creatinine may be due to the damage caused by CA on the function of kidneys. Uric acid, the metabolic end product of purine metabolism has been proven to be a selective antioxidant, capable of reacting with free

radicals and hypochlorous acid (33). The increased parameters return to normal by BM administration.

CA administration caused significant elevation in marker enzymes of AF, liver and heart. The significant increase in the activities of enzymes in liver and AF are due to the effect of CA on hepatocytes and possible transfer of the enzymes to AF. ALP is the prototype of hepatic marker enzyme that reflects the pathological alterations in bile flow (34). ALP and bilirubin used to evaluate chemically induced hepatic injury. Stabilization of ALP levels through the administration of BM. The increased protein content in AF may be due to leakage of Red blood corpuscles into AF. It clearly suggests that there is a decline in protein content in liver which could affect the metabolic activity of the liver. A protein being involved in the architecture and also in the physiology of the cell seems to occupy a key role in the cell metabolism (35). The fall in protein content during stress may be due to increased proteolytic

Table 4 : Volume of Amniotic fluid and weight of the embryo after CA treatment

S.No		Day of Exposure	CA concentration				
			Control	500	1000	2000	4000
1	Volume of AF	9 th day	2.12±0.02	1.94±0.02*	1.82±0.02*	1.62±0.02*	1.4±0.02*
2		12 th day	2.03±0.21	1.86±0.02	1.84±0.02	1.82±0.02	1.76±0.02
3		15 th day	1.96±0.02	1.82±0.02*	1.76±0.02*	1.72±0.02*	1.68±0.03*
4	Weight of Embryo	9 th day	10.82±0.02	10.64±0.02*	9.82±0.02*	9.32±0.02*	9.21±0.02*
5		12 th day	10.4±0.2	10.2±0.09	9.63±0.18*	9.25±0.06*	8.81±0.17*
6		15 th day	10.27±0.42	10.0±0.41	9.51±0.1**	9.14±0.14.0**	8.45±0.06*

* p<0.001 **p<0.05

activity and decreased anabolic activity of protein. The decreased protein content might also be due to tissue destruction by necrosis or disturbance of cellular fraction and consequent impairment in protein synthetic machinery (36). The reversal of protein levels were observed with BM administration. LDH, cytosolic enzyme involved in biochemical regulation reactions of the body tissues and fluids. In cytarabine mediated acute toxicity increased activity of LDH. BM extract ensured a rapid protection and maintained the levels of LDH.

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