ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 3, Issue 1, pp: (43-49), Month: January - March 2015, Available at: www.researchpublish.com

Andrographis Paniculata Nees's Protective Role on Cytarabine Induced Oxidative Damage in Chick Embryo

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Abstract: **A. paniculata are spread throughout south India and Srilanka which perhaps represent the centre of origin and diversity of the species. A. paniculata is of common occurrence in most of the places in our country including the plains and hilly areas. It is one of the important ingredients in Ayurvedic preparations recommended for fever and liver diseases. Andrographolide, chief constituent extracted from the leaves of the plant. The hepatoprotective action of andrographolide is related to activity of certain metabolic enzymes. Andrographis is the herb of Andrographis paniculata, a flowering plant in the Acanthaceae family. The active constituents of Andrographis paniculata are saponins, alkaloids, and phytosterols.**

1-β-arabinofuranosylcytosine (Cytosine arabinoside) (Ara-C) is the most important antimetabolite chemotherapeutic drug used for acute leukemia. In this study we examined the protective property of an ethanolic extract of Andrographolide paniculata on biochemical changes in chick embryo. CA caused biochemical changes in a concentration and time dependent manner in amniotic fluid, liver and heart tissues.

Keywords: **Andrographolide, Acanthaceae, saponins, phytosterols, hepatoprotective, Ara-C ,Amniotic fluid, Biochemical parameters and Chick embryo.**

I. INTRODUCTION

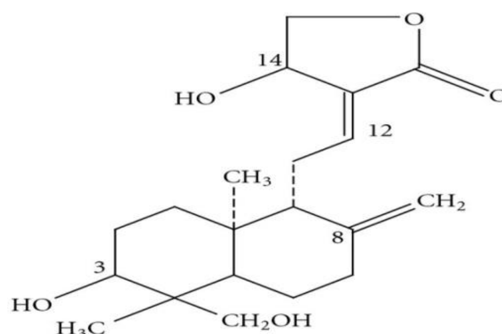
Andrographolide Paniculata is distributed in tropical Asian countries often in isolated patches. It can be found in a variety of habitats viz. plains, hill slopes, waste lands, farms, dry or wet lands, sea shore and even road sides. Native populations of A. paniculata are spread throughout south India and Sri Lanka which perhaps represent the centre of origin and diversity of the species. It is one of the important ingredients in Ayurvedic preparations recommended for fever and liver diseases. These include 'Liv 52' for treating hepatotoxicity (Dwivedi et al., 1987); 'TBR-002' for fever (Subramaniam et al., 1995), 'Kan Jang' for cold, flu and sinusitis (Hancke et al., 1995; Panossian et al., 2000). Despite its enormous medicinal and economic importance, attempts to cultivate A.paniculata have seldom been undertaken in any part of the country; hence local vaidyas as well as companies depend on wild sources for the supply of raw material. Practitioners of Chinese medicine believe that andrographis affects the large intestine, lung, stomach, bladder, and liver meridians, or energy pathways in the body. Major chemical ingredients include andrographan, andrographolide, neoandrographolide, paniculide A, 14-deoxy-11-oxyandrographolide, and beta- sitosterol.

Andrographolides paniculata Nees

II. ANDROGRAPHIS AND BETA GLUCAN

Medical practitioners have long understood the importance of the human body's own immune defences in fighting illness. Long before modern immunisation practices worked to arm the body against specific infections, traditional medical practitioners identified herbs which could stimulate the immune system, fortifying the body not just against specific infections, but against microbial invasion generally. Without realizing it, the Chinese have also been taking advantage of another effective immune stimulant for centuries. It is called beta glucan and it is found naturally in a variety drug of substances including oats, barley, and some fungi – including mushrooms eaten by the Chinese. The form of beta glucan now rising to prominence, however, is derived from the cell walls of baker's yeast. Herbs - like many manufactured drugs - contain highly complex molecules. That means that even when scientists know that they work, they don't always know exactly how.

In the case of andrographis, it has been established that its effect does not come from a direct antibacterial action - the herb itself does not kill bacteria. Extracts of andrographis, though, have been shown to stimulate powerful immune responses in living creatures. Beta glucan works by triggering the activating receptors of phagocytes in your body. When these cells are activated, they don't just get themselves ready to fight invaders - they stimulate our whole immune system to prepare itself. Activated phagocytes prompt the production of more white blood cells and because of the activity of beta glucan, those fresh bug-fighting cells are primed to defend your body.



Active ingredient –Andrographolides

III. MATERIALS AND METHODS

Preparation Of Plant Extract**-**The whole plant 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: All eggs of zero old days were cleaned with wet cotton and placed in sterile egg incubator maintained at 37 degree 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 Andrographis Paniculata 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 AP on 12th day and were intoxicated with a single dose of CA on the 15th day of experiment (i.e. 16th day).

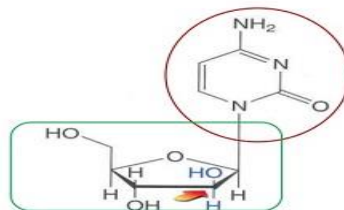
The embryos were exposed after 24h of CA administration, the AF and tissues of liver, heart and brain were collected for estimation of various biochemical parameters.

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

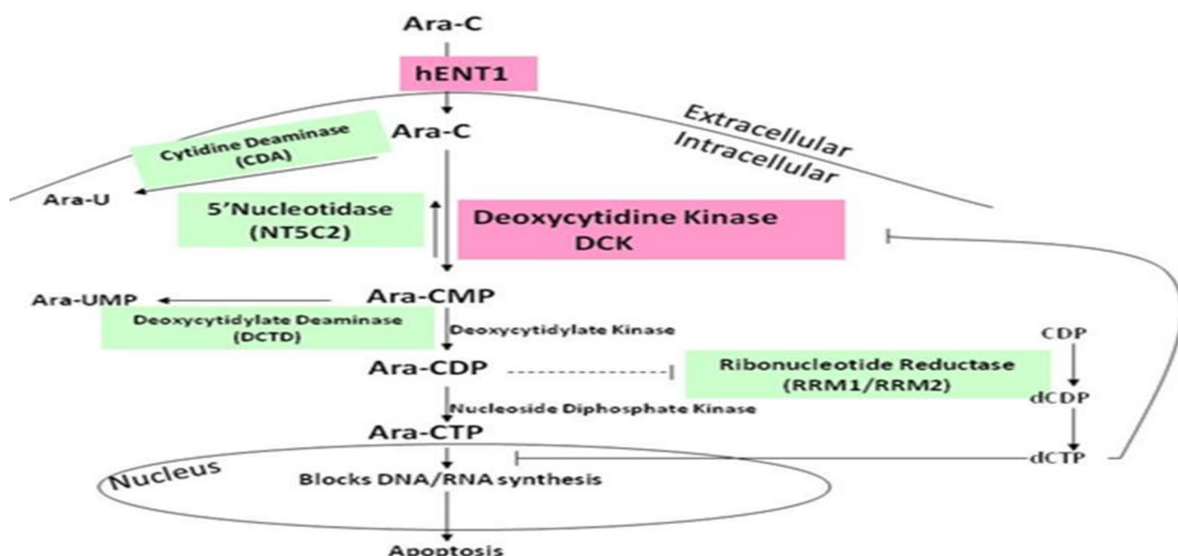
Tissue extract analysis: 10% homogenate of the embryonic liver, brain and heart was prepared by grinding in Tris HCl buffer, pH 7.4. The homogenate was centrifuged at 3000 rpm in Remi refrigerated centrifuged and the supernatant was used for the measurement of biochemical parameters. The protein content was estimated by Bradford method , glucose by O-toluidine method ,inorganic phosphorus by Fiske Subbarow method , urea by Natelson et al. , creatinine by Broad and Sirota ,uric acid by Caraway , cholesterol by Zlatkis et al. sodium and potassium concentration were measured using Flame photometer (ELICO). Marker enzymes of lactate dehydrogenase (LDH) by King method, alkaline phosphatase by King, alanine aminotransferase and aspartate aminotransferase (SGOT & SGPT) by King method.

CYTARABINE

- Ara-C / Cytosine Arabinoside



Mechanism of Cytarabine drug



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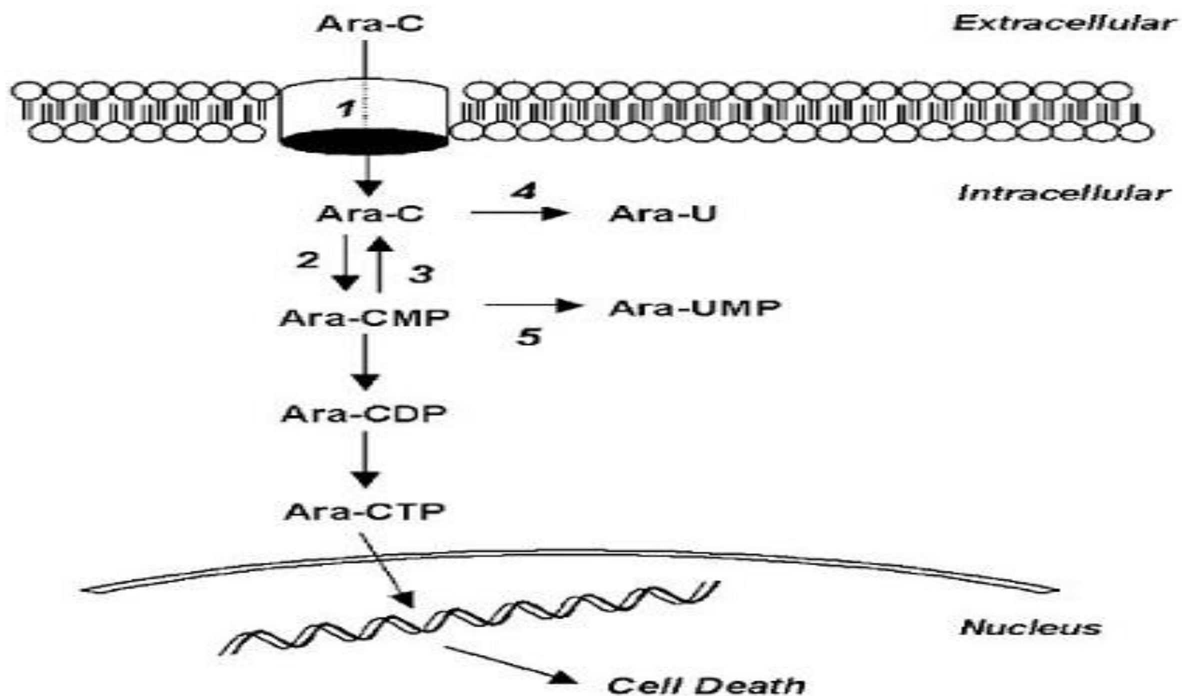

Step 1 above highlights the nucleoside transporter (hENT1) responsible for facilitation of cytarabine entry into the cell.

The next step involves phosphorylation to a monophosphate form, Ara-CMP, catalyzed by deoxycytosine kinase (dCK). An additional two phosphorylation steps are catalyzed by (a) (deoxy) cytidylate kinase (UMP-CMPK) and (b) nucleoside diphosphate kinases (NDKs).

These two phosphorylation steps result in the formation of araCTP, the active anticancer form. Apoptosis occurs upon araCTP incorporation into DNA during DNA synthesis.

Note that araCTP formation can be inhibited by pyrimidine nucleotidase 1 which attenuates the effect of deoxycytidine kinase.

Other enzymes can promote conversion of ara-C to other species, an effect that reduces AraCTP concentrations thereby potentially limiting its apoptosis-promoting action.For example, cytidinedeaminase and deoxycytidylatedeaminase are described as inactivating enzymes since they promote Ara-C conversion to Ara-U in the first instance and Ara-CMP, the monophosphate form, to Ara-UMP (uridine monophosphate) in the second case.3

IV. RESULTS

Table 1 : Biochemical components of Amniotic fluid in chick embryo

				CA + AP		
S.No	Biochemical Parameter	Control	CA (3 mg)	2 mg	4 mg	6 mg
1	Glucose (mg/dl)	51.5±1.6	61.3±0.1*	60.8±0.4*	57.9±0.6*	51.6±1.3**
2	Protein (mg/dl)	15.8±0.1	23.7±0.4*	20.05±0.1*	18.2±0.4*	15.3±0.1*
3	Uric acid (mg/dl)	8.2±0.08	13.9±0.3*	12.6±0.4*	10.1±0.1*	7.6±0.1*
4	Urea (mg/dl)	8.2±0.2	15.5±0.3*	15.2±0.1*	11.8±0.5*	7.35±3.0*
5	Creatinine (mg/dl)	0.29±0.1	0.8±0.02*	0.5±0.05*	0.37±0.02*	0.24±0.01*
6	Sodium (mEq/L)	131.2±2.2	121.0±1.4*	125.1±0.7*	129.4±0.8*	130.3±0.1**
7	Potassium (mEq/L)	2.5±0.4	3.7±0.4*	3.4±0.1*	2.9±0.08	2.07±0.08**
8	Inorganic Phosphorus (mg/dl)	0.3±0.05	0.29±0.05*	0.55±0.05*	0.52±0.01*	0.2±0.01*
	* p<0.001 **p<0.05					

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 Andrographis Paniculata (AP) afforded a significant protection against CA induction. Table 1 represents the biochemical parameters in chick amniotic fluid in controls and CA (CA+AP) treated embryos. AP 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+AP treatment a significant decrease in glucose, protein, urea, uric acid and creatinine, inorganic phosphorus levels were observed. Glucose levels were significantly decreased ($p<0.05$) with 6mg AP treatment

The cholesterol, sodium, potassium levels were significantly decreased with CA treatment, where as increased in AP 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+AP treated ones. Serum SGOT and SGPT activities were significantly increased ($p<0.001$) with CA treatment, whereas significantly decreased ($p<0.001$) with AP treatment.Table 2 depicts the treatment with CA elevated biochemical parameters of ALP, SGOT, SGPT, and LDH were observed. The levels of protein, and inorganic phosphorus were decreased. The altered biochemical features were significantly brought towards normalization by treatment with AP 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, and cholesterol were observed. The enzymatic levels of LDH significantly increased ($p<0.001$) with CA treatment compared to control. The altered protein, inorganic phosphorus, and cholestrol levels were observed with AP treated embryos. The enzymatic levels of LDH level were also altered with AP treated embryos compared to CA treated ones.

Table 2 : Biochemical components of liver tissue in chick embryo

				CA + AP		
S.no	Parameter	Control	CA (3.0 mg)	2 mg	4 mg	6 mg
1	ALP #	8.1±0.1	13.2±0.1*	12.1±0.1*	9.8±0.3*	9.07±0.08*
2	SGOT# #	9.8±0.3	18.4±0.1*	14.9±0.2*	12.3±0.1*	10.5±0.2**
3	SGPT# #	6.2±0.08	9.4±0.1*	8.5±0.1*	7.2±0.3*	6.7±0.1*
4	LDH~	72.6±0.1	86.4±0.2*	80.5±0.1*	78.7±0.4*	74.5±1.05**
5	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*

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

Table 3: Biochemical components of Heart tissue in chick embryo

				CA +AP		
S.No	parameter	control	CA (3mg)	2mg	4mg	6mg
1	Cholesterol#	24.5 ± 2.1	14 ± 1*	16.1 ±0.5*	20.1 ± 0 .2*	21.9 ± 0.8*
2	Protein#	28.3 ± 0.08	19.5 ± .1*	20.3 ± 0.3*	25.1 ± 0 .1*	26.8 ± 0.5**
3	inorganic phosphorous#	0.9 ± 0.08	0.8 ± .08*	0.79 ± 0.2*	0.84 ±0 .9*	0.88 ± 0.2*

* p<0.001 **p<0.05
# (mg/100mg dry weight)

V. DISCUSSION

In this work the administration of CA caused significant biochemical changes in AF, liver and heart tissues of chick embryo. The biochemical changes in AF, liver and heart would be well used to hextrapolate 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 were significantly increased with CA treatment in AF compared to controls. The change in glucose level is an indication of alterations on carbohydrate metabolism. The reversal of increased glucose returned to normal by AP 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. The increased parameters return to normal by AP 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. ALP and bilirubin used to evaluate chemically induced hepatic injury. Stabilization of ALP levels through the administration of AP. 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.

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

			CA concentration				
S.No		Exposure Day	Control Mean±S.D	500	1000	2000	4000
1	Volume of AF	9th day	2.12±0.02	1.93±0.02*	1.81±0.02*	1.65±0.02*	1.39±0.02*
		12th day	2.03±0.21	1.84±0.02	1.82±0.02	1.81±0.02	1.73±0.02
		15thday	1.96±0.02	1.85±0.02*	1.73±0.02*	1.70±0.02*	1.66±0.03*
2	Weight of embryo	9th day	10.82±0.02	10.69±0.02*	9.68±0.02*	9.23±0.02*	9.191±0.02*
		12th day	10.4±0.2	10.1±0.09	9.58±0.18*	9.18±.06*	8.88±0.17*

* $p<0.001$ ** $p<0.05$

The fall in protein content during stress may be due to increased proteolytic 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. The reversal of protein levels were observed with AP administration. LDH, cytosolic enzyme involved in biochemical regulation reactions of the body tissues and fluids. In cytarabine mediated acute toxicity increased activity of LDH. AP extract ensured a rapid protection and maintained the levels of LDH.

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