

International Journal of Drug Research and Technology

Available online at <http://www.ijdr.com>

Original Research Paper

EVALUATION OF ANTI-GENOTOXIC ACTIVITY OF APOCYNIN AGAINST CYCLOPHOSPHAMIDE-INDUCED DAMAGES IN BONE MARROW CELLS OF MICE AND ROOT MERISTEM CELLS OF *ALLIUM CEPA* L

G. Hema^{1,2*}, D. Subbarao² and B.S. Thippeswamy³,

¹Department of Biotechnology, Maharani' Science College for Women Bangalore, India

²Jawahar Lal Nehru Technological University, Anantapur, Andhra Pradesh, India

³Sree Siddaganga College of Pharmacy, B.H. Road, Tumkur, Karnataka, India

ABSTRACT

Increased exposure to environmental pollutants, industrial chemicals and cytotoxic substances can lead to mutagenic events in DNA and carcinogenesis. Some naturally occurring compounds are known to inhibit mutagenesis and carcinogenesis *in vitro* and *in vivo*. Apocynin is a major constituent of extracts of Himalayan herb *Picrorhiza kurroa*, used traditionally to treat asthma and inflammation and is a strong NADPH-oxidase inhibitor. Our studies aimed at assessing the genotoxic and antigenotoxic properties of Apocynin against cyclophosphamide induced genotoxicity in bone marrow of mice and root tip meristematic cells of *Allium cepa*. The results of our experiments indicate the ability of Apocynin to significantly lower the incidence of micronucleated PCEs and chromosomal aberrations in the test systems.

Keywords: Genotoxicity, Apocynin, Micronucleated PCEs, Chromosomal aberrations, Cyclophosphamide.

INTRODUCTION

Humans are continuously exposed to several environmental pollutants and industrial chemicals that lead to the development of different kinds of cancer. The exposure to these agents is often unavoidable and creates a great risk to human health. The human body is inherently equipped with enzymatic detoxification processes to defend against these toxic agents. But increased or repeated exposure to hazardous chemicals can lead to mutagenic events as in the case of cancer chemotherapy. An alternative approach is to help the host organism to oppose the attack of mutagens and carcinogens by supplementing the diet with chemoprotective agents (De Flora and Ferguson, 2005). Plants produce secondary metabolite in response to environmental stresses and these are called as phytochemicals. Thousands of these phytochemicals have been identified and when consumed in human diet may

affect chronic disease risk. Literature review suggests that plant extracts and their active principles have proved to contain variety of antimutagenic, antigenotoxic potential (Scassellati-Sforzolini et al., 1999; Pasquini et al., 2002; Hayder et al., 2004; Bhourri et al., 2010; Steinmetz and Potter, 1991 and Gupta et al., 2010). One of the ways by which phytochemicals can protect against cell proliferation is by destroying reactive oxidative species (ROS) that initiate carcinogenesis through oxidative damage of DNA) (Williams et al., 1989) or suppress their production. Apocynin (4-hydroxy-3-methoxy-acetophenone) is a constituent of the Himalayan herb *Picrorhiza kurroa* Royle (Scrophulariaceae) that is well known in traditional Indian medicine (Ayurveda). The plant has been widely used in traditional system medicine for the treatment of asthma, fever, inflammation and rheumatoid

arthritis (Stefanska and Pawliczak, 2008). It has been used as an efficient inhibitor of the Nicotinamide Adenine Dinucleotide Phosphate (NADPH)-Oxidase in many experimental models such as colitis, rheumatoid arthritis, ischemia reperfusion and lung injury. Apocynin is an acetophenone to which a range of biological activities is attributed. It is a pro drug that is converted by peroxidase mediated oxidation to a dimer, which has been shown to be more efficient than apocynin itself (Rhian MT, 2008). Phytochemicals with pronounced antioxidant and anti-inflammatory effects are anticipated to act as anti-tumor promoters (Surh, 2002). Cyclophosphamide (CP), an alkylating agent has been used for treating malignant as well as non-malignant disorders since a long time. Its metabolites are toxic to cancer cell as well as normal cells also (Fraiser *et al.*, 1991), primarily causing DNA damage by oxidative stress (Brookes, 1990). Oxidative stress can damage DNA, lipids, proteins, and carbohydrates leading to impaired cellular function and enhanced inflammatory reactions (Wang *et al.*, 2006). Based on these findings, the aim of the present study was directed to investigate the protective effects of Apocynin on genotoxicity induced by cyclophosphamide in mice and chromosomal aberrations using *Allium cepa* root tip assay.

MATERIALS AND METHODS

Chemicals

Cyclophosphamide (CP) was purchased from local licensed outlets (Brand name-Uniphos 500, United Biotech, India (Batch No.UPDJ3B7-Oct, 2013). Apocynin was purchased from Natural Remedies Ltd., Bangalore (batch No.T12G190-2014). All other chemicals and stains used in the assay were of analytical grade.

Animals

Eight week-old healthy Swiss albino mice of either sex, weighing 25 ± 5 g were purchased from Venkateshwara enterprises, Bangalore, India. Animals were maintained under conventional laboratory conditions, at temperature $25 \pm 2^\circ\text{C}$, and a 12 h natural light period. Commercial pellet diet (Lipton India) and drinking water were provided

ad libitum. Animal experiments were carried out after obtaining the clearance from the institutional animal ethical committee.

Experimental Design

Animals were divided into five groups of 6 mice each were formed and subjected to various treatments as follows. Group 1: Normal control-received the vehicle (saline) intraperitoneally for 5 days 10 ml/Kg body weight. Group 2: Plant active principle control-received Apocynin 200 $\mu\text{g}/\text{kg}$ body weight for 5 days i.p. Group 3: Clastogenic control-received CP alone 50 mg/kg body weight i.p. in a single dose. Group 4: Treated with Apocynin 100 $\mu\text{g}/\text{kg}$ body weight for 5 days and 1 hr after the last dose given a single dose of CP 50 mg/kg body weight i.p. Group 5: Treated with Apocynin 200 $\mu\text{g}/\text{kg}$ body weight for 5 days and 1 hr after the last dose given a single dose of CP 50 mg/kg body weight i.p. The animals were sacrificed after 24 of CP administration by cervical dislocation under light anesthesia. Before sacrifice the animal's blood samples were collected for the estimation of WBCs, RBCs and Hemoglobin content. The femur and tibia were excised for collection of Bone marrow and estimation of mnPCE's.

Micronucleus Assay

Micronucleus test was performed by counting the number of micronuclei present in the bone marrow of control and pretreated mice. Bone marrow slides were prepared as per the method of Hayashi with little modification (Hayashi *et al.* 1994). Marrow suspension from femur and tibia bones prepared in 5% bovine serum albumin (BSA) was centrifuged at 1000 rpm and the pellet was re-suspended in BSA solution. A drop of this suspension was placed on a clean glass slides and smears were prepared and air-dried. The slides were coded to avoid observed bias. The slides were fixed in methanol, stained for 5 min in May-Gruenwald solution and for 10 min in Giemsa respectively. Then slides were rinsed in distilled water, cleaned on the back side with filter paper and then dried on the slide warmer. 1000 polychromatic erythrocytes or PCEs per animal were scanned for the presence of micronucleus.

Allium cepa Root Tip Assay for Chromosomal Aberrations

The Allium test was performed according to Solange and Laughinghouse, 2010, with slight modifications. Onion bulbs were purchased and sundried for a week. Healthy bulbs without fungal attack of approximately 20 g were chosen, the dried outer scales were removed and the dried roots were cut retaining the root primordia. The bulbs were placed in disposable plastic cups immersing the root primordia in plain drinking water for three days allowing roots to emerge, at room temperature (Around 28°C). Water was changed periodically every 24 hrs. When the rootlets reached a length of 2-3 cm, they were exposed to different treatments mentioned as follows: Group-1 Negative control (plain drinking water), Group-2 Positive control (Cyclophosphamide 2 mg/ml in water), Group-3 (Cyclophosphamide 2 mg/ml + Apocynin 100 µg/ml in water), Group-4 (Cyclophosphamide 2 mg/ml + Apocynin 200 µg/ml in water), Group-5 and 6 treated with Apocynin alone 100 and 200 µg/ml in water respectively. After 24 hrs of exposure to the treatments, the roots were rinsed with plain water and root tips of about 1cm length were collected. The root tips were fixed in Carnoy's fixative (3:1 ethyl alcohol: acetic acid) for 24 hrs and thereafter preserved in 70% alcohol. The preserved root tips were then subjected to microscopic cytogenetic analysis. Root tips of about 2 mm length were hydrolysed in 1N HCl and then stained with Aceto-orcein. Stained tips were squashed on glass slides under cover slips to spread the cells. Dividing cells in all stages of mitosis i.e., prophase, metaphase, anaphase and telophase were counted under DeWinter camera microscope at 400X magnification. Two root tips showing at least 10 dividing cells per 1000 cells were selected from each bulb (replication) for analysis. 1000 cells per root tip were screened totalling to 6000 cells per treatment.

The mitotic index was calculated as follows (Akinboro *et al.*, 2011)-

$$MI = n_d / n_t \times 100\%$$

n_d -- number of dividing cells

n_t -- total number of cells

The number of cells showing mitotic abnormalities like lagging chromosomes, spindle abnormalities, adherent nucleus, anaphasic bridges and broken chromosomes were also counted per 1000 cells in each root tip.

The frequency of chromosomal aberrations was calculated as follows:

$$fCA = n_a / n_t \times 100\%$$

Where n_a is the number of aberrant cells.

RESULTS

Effect of Apocynin on Cyclophosphamide Induced Micronuclei PCE

Administration of Apocynin alone in the dose of 200 µg/kg bodyweight of experimental mice did not lead to significant formation of micronuclei in polychromatic erythrocytes. CP (50 mg/kg body weight) induced significant number of mnPCEs as compared to the normal control in the bone marrow cells of the mice. Treatment with CP50 + Apocynin at concentrations of 100 and 200 µg/kg bodyweight of mice showed significant reduction in the formation of micronucleated polychromatic erythrocytes when compared to animals exposed only to CP (Table-1).

Effect of Apocynin on WBCs, RBCs and Haemoglobin Level in Cyclophosphamide Treated Mice

CP significantly lowered the total WBC count as compared the normal control while Apocynin alone does not have any significant lowering effect on the number of WBC as compared to normal control. But in animals treated with Apocynin along with CP, the WBC count was significantly increased when compared to animals treated with CP alone. The total RBCs and haemoglobin content in the various experimental groups are not significantly altered by the treatments (Table-1).

Effect of Apocynin on Cyclophosphamide Induced Chromosomal Aberration in Onion Roots

Mitotic Index

The mitotic index was highly decreased by treatment with CP as compared to negative control, plain drinking water. Presence of

Apocynin along with CP reversed the action of CP by significantly increasing the MI as compared to CP alone. The action of Apocynin was dose dependent with 200 µg/ml being more significant. Apocynin alone did not modify the mitotic index (Table-2).

Frequency of Chromosomal Aberrations

The frequency of chromosomal aberrations significantly very high in the cyclophosphamide treated positive control as compared to plain drinking water. The same was very significantly lowered by inclusion of Apocynin at a concentration of 200 µg/ml along with CP. Apocynin showed a dose dependent reduction in the fCA with 200 µg/ml of Apocynin being more effective than 100microgram/ml (Table-2 Figure-1).

DISCUSSION

The mammalian *in vivo* micronucleus test is used for the detection of damage caused by the test substance to the chromosomes or the mitotic apparatus of erythroblasts sampled in the bone marrow (OECD guidelines, 1997; Heddle and Salamone, 1981). The appearance of micronuclei following Cyclophosphamide exposure was majorly due to chromosomal damage (Muller and Streffer, 1994). The mutagenicity of CP is attributed to the formation of Cytotoxic phosphoramidate mustard which is capable of inducing DNA cross links and strand lesions. (Hales, 1982). CP has been consistently shown to cause genotoxic effects in different *in vitro* and *in vivo* test systems. (Mishra *et al.*, 2013 and Gupta *et al.*, 2010). CP also shows significant immunosuppressive effect (Wojcik and Dabkowska). In the current study the administration of CP alone to experimental animals has shown significantly higher number of micronucleated polychromatic erythrocytes as compared to normal control and Apocynin. Apocynin along with CP resulted in statistically significant reduction of the number of mnPCEs in the bone marrow compared to CP. Apocynin is a proven inhibitor of NADPH oxidase (Stefanska and Pawliczak, 2008) with strong free radical scavenging property and hence, it could have

nullified the DNA damaging effects of CP induced free radicals (Stankiewicz, A, 2002) leading to reduction in formation of micronuclei. CP induced significant reduction in the total WBC count of the treated animals when compared to normal control which is also in agreement with previous literature (Merwid-Lad *et al.*, 2011; Gupta *et al.*, 2010). In mice treated with Apocynin along with CP, there is a significant increase in the WBC count compared to the CP alone. This is because of the potential of Apocynin to prevent the production of superoxide radicals in WBC and neutrophilic granulocytes and thus mitigate the oxidative damage to WBC caused by CP.

Impairment of immune system is a major side effect of chemotherapeutic agents like CP. Restoration of the immune activity in the patients is very important. Our results indicate that Apocynin can support the restoration of immune functions. There is no significant effect of any of the treatments on the total RBC and haemoglobin in the experimental animals.

The *Allium cepa* assay is a well known and widely used plant based *in vivo* test system used to assess the genotoxic and antigenotoxic properties of substances (Donatella, F *et al.*, 2007; Solange and Laughinghouse, HD, 2012). Several workers have established the utility of this system in evaluating the toxicity/genotoxicity of drugs, pesticides and environmental pollutants (Qari, SHM, 2010; Akinboro, A, 2011) Apocynin also led to significant reduction in the occurrence of chromosomal aberrations and increase in the rate of mitosis in onion root tip meristematic cells in the presence of CP. The absence of any genotoxic effects of Apocynin at the tested concentrations is of importance. Oxidative DNA damage is an important cause of pathological processes like aging and cancer. It can be a result of normal metabolism in which ROS are formed or induced by factors like UV light, ionizing radiations, industrial chemicals and genotoxic agents. (Bringmann *et al.*, 2001). Agents which can counteract this damage are of great significance in chemoprevention of cancer. Plant species rich in Apocynin have been used as anti-

asthmatic, anti-arthritic agents, anti-inflammatory agent and in treatment of liver and heart problems, jaundice and bowel diseases. The

results of our studies indicate that Apocynin could be a potential antigenotoxic agent suitable for use in chemotherapy cases.

Table 1: Effect of Apocyanin on Cyclophosphamide Induced Micronucleus Test in Mice

Treatment	Mean no. of mnPCE/1000 PCE	WBC count (cells/cu.mm)	RBC count (million cells/cu.mm)	Haemoglobin Content ($\mu\text{g}/\text{cu.mm}$)
Normal control	4.00 \pm 0.81	8669 \pm 366.9	7.23 \pm 0.465	12.61 \pm 0.301
Apocyanin 200	11.50 \pm 1.19	7418 \pm 390.9	7.78 \pm 0.436	13.69 \pm 0.864
Cyp 50 mg/kg i.p.	35.75 \pm 3.0***a	3928 \pm 257.2***a	7.615 \pm 0.33	10.56 \pm 1.163
Cyp 50+Apo 100 $\mu\text{g}/\text{kg}$ p.o.	14.00 \pm 2.0**b	6676 \pm 476.3**b	7.465 \pm 0.33	8.708 \pm 0.789
Cyp 50+Apo 200 $\mu\text{g}/\text{kg}$ p.o.	7.25 \pm 2.8***b	6615 \pm 678.2**b	8.513 \pm 0.35	10.75 \pm 0.263

[Values expressed are mean \pm SEM n=06] ** p<0.01, ***p<0.001 a-when compared with normal control b- when compared with positive control (CP) control.

Results of *Allium Cepa* Root Tip Assay

Table 2: Frequency of Chromosomal Aberrations and Mitotic Index

Groups	Replication	fCA	Mean \pm SEM	MI	Mean \pm SEM of MI
1	1	0	0	20.35	19.33 \pm 2.322
	2	0		22.75	
	3	0		14.90	
2	1	0.45	0.4833 \pm 0.088***	3.30	3.567 \pm 0.2667***
	2	0.65		4.10	
	3	0.35		3.30	
3	1	0.1	0.1667 \pm 0.0441*	9.45	9.867 \pm 0.7928*
	2	0.15		11.40	
	3	0.25		8.75	
4	1	0	0.10 \pm 0.0763**	15.35	15.17 \pm 0.4567***
	2	0		14.30	
	3	0.05		15.85	
5	1	0.1	0.0833 \pm 0.016	19.00	16.83 \pm 1.084
	2	0.05		15.80	
	3	0.1		15.70	
6	1	0	0.050 \pm 0.0288	16.95	16.85 \pm 0.350
	2	0.1		17.40	
	3	0.05		16.20	

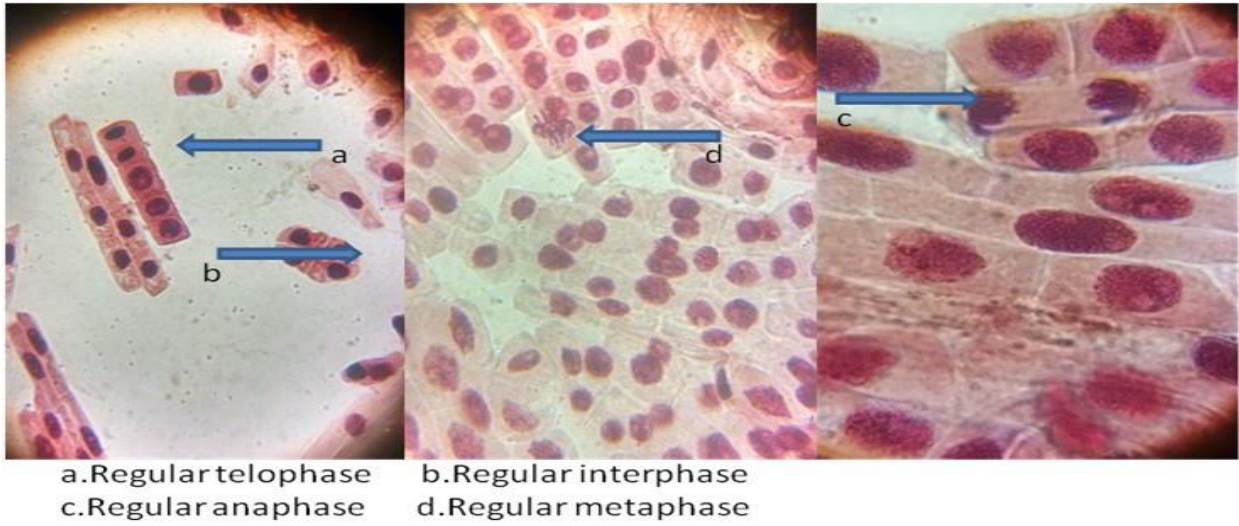


Figure1: Shows different phases of regular mitosis in onion root tip meristems

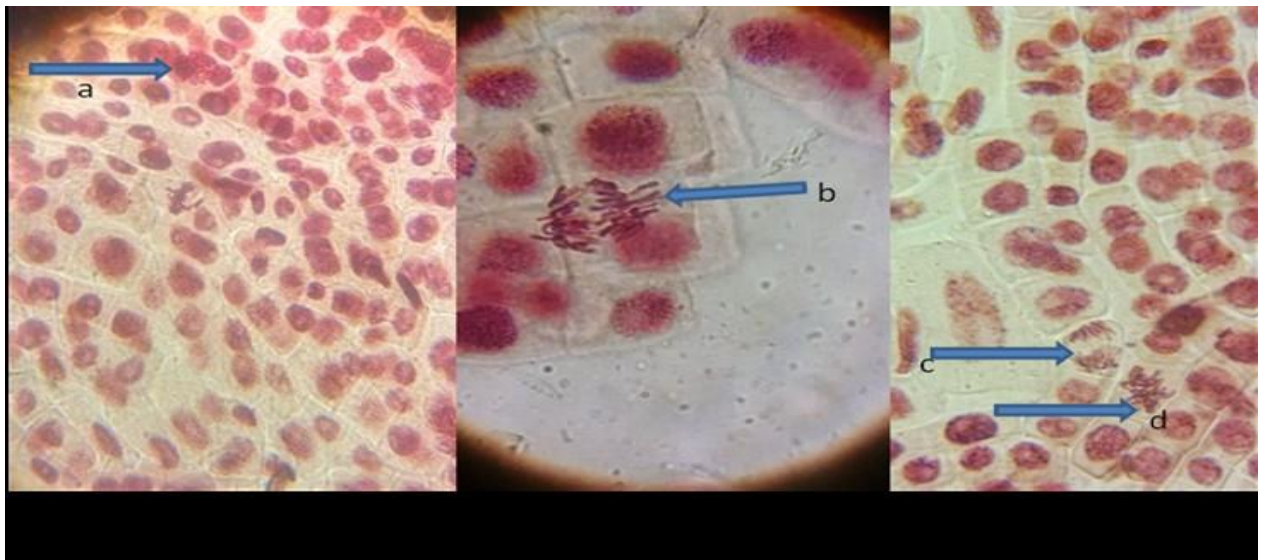


Figure 2: Shows different types of mitotic abnormalities in onion root tips from bulbs treated with CP.

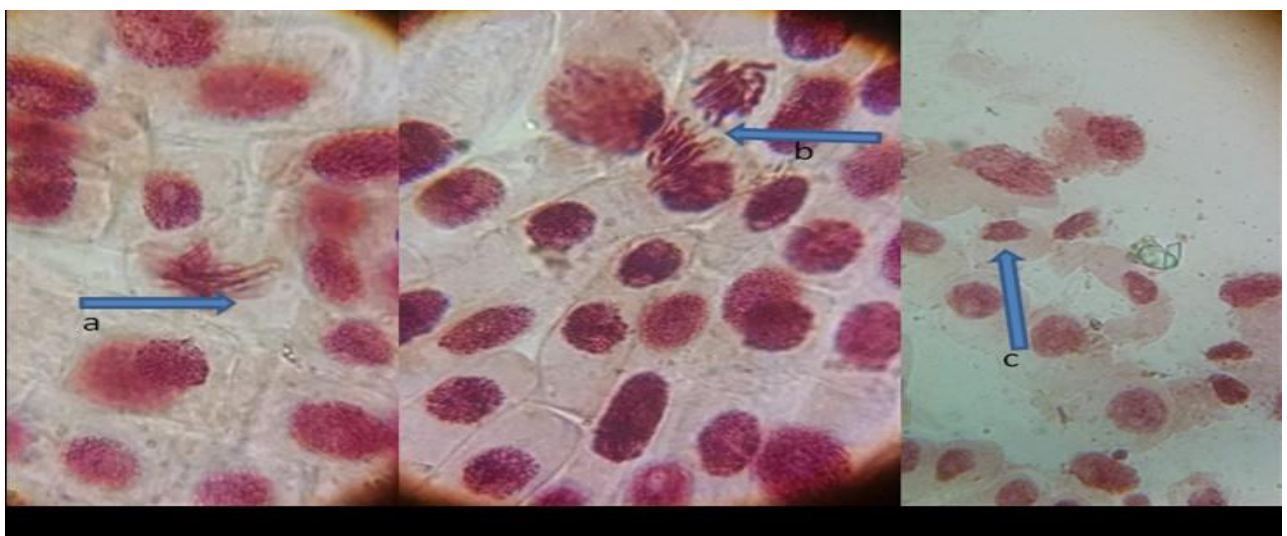


Figure 3 : Shows different types of mitotic abnormalities in onion root tips from bulbs treated with CP

REFERENCES

- De Flora, S and Ferguson, LR (2005), "Overview of mechanisms of cancer chemopreventive agents", *Mutation Research*, 591, 8-15.
- Scassellati-Sforzolini, G; Villarini, LM; Moretti, LM; Marcarelli, LM; Pasquini, R and Fatigoni, C (1999), "Antigenotoxic properties of *Terminalia arjuna* bark extracts", *Journal of Environmental Pathology Toxicology and Oncology*, 18, 119-125.
- Pasquini, R; Scassellati-Sforzolini, G; Villarini, M; Moretti, M; Marcarelli, M; Fatigoni, C; Kaur, S; Kumar, S and Grover, IS (2002), "In vitro protective effects of *Terminalia arjuna* bark extract against the 4-nitroquinoline-N-oxide Genotoxicity", *Journal of Environmental Pathology Toxicology and Oncology*, 21, 33-44.
- Hayder, N; Abdelwahed, A; Kilani, S; Ammar, RB; Mahmoud, A; Ghedira, K and Chekir-Ghedira, L (2004), "Anti-genotoxic and free radical scavenging activities of extracts from (Tunisian) *Myrtus communis*", *Mutation Research*, 564, 89-95.
- Bhourri, W; Derbel, S; Skandrani, I; Boubaker, J; Bouhleb, I; Mohamed, BS; Kilani, S; Mariotte, AM; Dijiux-franca, MG; Ghedira, K and Chekir-Ghedira, L(2010), "Study of genotoxic, antigenotoxic and antioxidant activities of the Digallic acid isolated from *Pistacia lentiscus* fruits", *Toxicology In Vitro*, 24,509-515.
- Steinmetz, KA and Potter, JD (1991), "Vegetables, fruit and Cancer Epidemiology", *Cancer Causes Control*, 2, 325-357.
- Gupta, A; Gautam, MK; Singh, RK; Vijay Kumar, M; Rao, ChV; Goel, RK and Anupurba, S (2010), "Immunomodulatory effect of *Moringa oleifera* Lam. extract on Cyclophosphamide induced toxicity in mice", *Indian Journal of Experimental Biology*, 48, 1157-1160.
- Williams, DE; Dashwood, RH; Hendricks, JD and Bailey, GS (1989), "Anticarcinogens and tumor promoters in foods", In: *Food Toxicology-A Perspective on Relative Risks*, Taylor, SL and Scanlan, RA (Eds.), *Marcel Dekker*, New York, 101.
- Stefanska, J and Pawliczak, R (2008), "Apocynin: Molecular Aptitudes", *Mediators of Inflammation*, Hindawi Publishing Corporation.
- Rhian, MT (2008), "Apocynin,NADPH oxidase and Vascular cells", *Hypertension*,51,172-174
- Surh, YJ (2002), "Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review", *Food and Chemical Toxicology*, 40, 1091-1097.
- Fraiser, LH; Kanekal, S and Kehrer, JP (1991), "Cyclophosphamide toxicity", *Drugs*, 42,781-795.
- Brookes, P (1990), "The early history of Biological alkylating agents", *Mutat. Res*, 233, 3-14.
- Wang, L; Tu, YC; Lian, TW; Hung, JT; Yen, JH and Wu, MJ (2006), "Distinctive antioxidant and anti-inflammatory effects of flavonols", *Journal of Agricultural and Food Chemistry*, 54, 9798-9804.
- Hayashi, M; Tice, RR; MacGregor, JT; Anderson, D; Blakey, DH; Kirsch-Volders, M; Oleson, FB Jr; Pacchierotti, F; Romagna, F; Shimada, H; Sutou, S and Vannier, B (1994)," In vivo rodent erythrocyte micronucleus assay", *Mutat. Res.*, 312 (3),293-304.
- Solange, BT and Laughinghouse, HD (2012), "Bio indicator of genotoxicity: The *Allium cepa* test", In: *Environmental Contamination*, Dr. Jatin Srivastava (Ed.), ISBN: 978-953-51-0120-8, In Tech, available from: <http://www.intechopen.com/books/environmental-contamination/bioindicator-of-genotoxicity-the-allium-cepa-test>
- OECD (1997), "OECD Guidelines for the testing of Chemicals,No.475: Mammalian Erythrocyte Micronucleus Test".
- Heddle, JA and Salamone, MF (1981), "Chromosomal Aberrations and Bone Marrow

- Toxicity”, *Env. Health. Perspective*, 39, 23-27.
19. Muller, WU and Streffer, C (1994), “Micronucleus Assays”, In: Obe, G(Ed.) *Advances in Mutagenesis Research*, Vol. 5, Springer, New York, 1-134.
20. Hales, BE (1982), “Comparison of the mutagenicity and teratogenicity of Cyclophosphamide and its active metabolites, 4-hydroxycyclophosphamide, phosphoramidate mustard and acrolein”, *Cancer Res.*, 42, 3016-3021.
21. Mishra, A; Gupta, V; Nagar, H and Shrivastav, V (2013), “Protective effects of *Murraya Koengii* (Curry leaf) leaves extract against genotoxicity induced by Cyclophosphamide in mouse bone marrow cells”, *Global Veterinaria*, 10 (2), 128-133.
22. Wojcik, R and Dabkowska, A (2010), “The effect of Cyclophosphamide on the selected parameters of immunity in rats”, *Centr. Eur. J Immunology*, 35 (1), 1-9.
23. Stankiewicz, A; Skrzydlewska, E and Makiela, M (2002), “Effects of amifostine on liver oxidative stress caused by Cyclophosphamide administration to rats”, *Drug Metabol Drug Interact*, 19, 67-82.
24. Merwid-Lad, A; Trocha, M; Chlebda, E; Sozanski, T; Magdalan, J; Ksiadzyna, D; Piesniewska, M and Szelag, A (2011), “The effects of Morin, a naturally occurring flavonoid, on Cyclophosphamide-induced toxicity in Rats”, *Adv. clin. Exp. Med.*, 20 (6), 683-690.
25. Donatella, F; Ilaria, Z; Claudia, Z; Elisabetta, C and Silvano, M (2007) “*Allium cepa* chromosome aberration and micronucleus tests applied to study genotoxicity of pesticide-treated vegetable extracts”, *Food Additives and Contaminants*, 24 (06), 561-572.
26. Qari, SHM (2010), “DNA-RAPD fingerprinting and Cytogenetic screening of genotoxic and antigenotoxic effects of aqueous extracts of *Costus Speciosus*(Koen.)”, *JKAU: Sci.*, 22 (1), 133-152
27. Akinboro, A; Mohamed, KB; Asmawi, MZ; Sulaiman, SF and Sofiman, OA (2011), “Antioxidants in aqueous extract of *Myristica fragrans*(Houtt.) suppress mitosis and Cyclophosphamide-induced chromosomal aberrations in *Allium cepa* L. cells”, *J Zhejiang Uni-Sci B (Biomed and Biotechnol)*, 12 (11), 915-922.
28. Bringmann, G; Monchbach, M; Feineis, D; Faulhaber, K and Ihm, H (2001), “Studies on single strand scissions to cell free plasmid DNA by the dopaminergic neurotoxin dTaCloT (1-trichloromethyl-1, 2, 3, 4-tetrahydro-h-carboxline)”, *Neuroscience Letters*, 304, 41-44.

Correspondence Author:

G. Hema

Department of Biotechnology, Maharani' Science College for Women Bangalore, India

Jawahar Lal Nehru Technological University, Anantapur, Andhra Pradesh, India



Cite This Article: G, Hema; D, Subbarao and BS, Thippeswamy (2015), “Evaluation of anti-genotoxic activity of apocynin against cyclophosphamide-induced damages in bone marrow cells of mice and root meristem cells of *Allium cepa* L”, *International Journal of Drug Research and Technology*, Vol. 5 (4), 149-156.