ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Cyclophosphamide-induced oxidative stress in brain: Protective effect of *Garcinia indica* fruit extract

Tushrendra Singh[1]*, S. B. Kasture[2], P.K. Mohanty[1], Yusuf Jaliwala[1], Manvendra Singh Karchuli[2], Abhisek Agarwal[1] and Yashraj Yadav[1]

1, VNS Institute of Pharmacy, Bhopal, (M.P.) - India

2, Pinnacle Biomedical Research Institute, Bhopal, (M.P.) – India

Abstract

This study sought to characterize the distribution of phenols and antioxidant activities in *Garcinia Indica* fruit extract and their inhibition of cyclophosphamide-induced oxidative stress in rat's brain. The total Phenol content and antioxidant activities of *Garcinia Indica* fruit extract were determined *in vitro* and *in vivo*. The Results of the study revealed that intraperitoneal administration of cyclophosphamide (75mg/kg of body weight) caused a Significant increase (P<0.05) in the malondialdehyde (MDA) content of the brain; however, there was a significant Decrease (P<0.05) in the brain MDA content, in those of rats treated with *Garcinia Indica* fruit extract showed a higher Inhibitory effect. In addition, treatment of the *Garcinia Indica* fruit extract also caused a dose-dependent inhibition of Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase and total bilirubin; likewise, treatment of the *Garcinia Indica* fruit extract inhibited MDA production. The higher Inhibition of oxidative stress in brain and serum enzymes and metabolites by the *Garcinia Indica* fruit extract could be attributed to its significantly higher (P<0.05) total phenol content, reducing power and free-radical scavenging ability. Therefore, treatment of *Garcinia Indica* fruit extract could prevent cyclophosphamide-induced oxidative stress in brain; although the *Garcinia Indica* fruit extract is a better protectant.

Key-Words: *Garcinia Indica*, Phenol, DPPH, Antioxidant, Cyclophosphamide, Brain.

Introduction

Oxidative stress is created when excessive free radicals react with proteins, cell walls and DNA, causing damage to cell structures and thereby leading to degenerative diseases. The brain and nervous system are particularly vulnerable to oxidative stress due to limited antioxidant Capacity (Shulman et al., 2004). The brain makes up about 2% of a person's mass but consumes 20% of their metabolic oxygen. The vast majority of this energy is used by the neurons (Shulman et al., 2004). Some brain cells, like neurons, cannot make glutathione, but instead rely on surrounding astrocyte cells to provide useable glutathione precursors, because the brain has limited access to the bulk of antioxidants produced by the body, neurons are the first cells to be affected by a shortage of antioxidants, and are most susceptible to oxidative stress (Perry et al., 2004).

*** Corresponding Author:**
E-mail: singhtushrendra@gmail.com

The most likely and practical way to fight against degenerative diseases is to improve body antioxidant status, which could be achieved by higher consumption of food rich in antioxidant phytochemicals. Foods from plant origin usually contain natural antioxidants that can scavenge free radical. The human body is equipped with an antioxidant defense system that deactivates these highly reactive free radicals. This includes antioxidants enzymes (made in the body) and antioxidant phytochemicals that soak up the excess reactivity that these free radicals have, turning them to harmless particles or waste products that can be get rid of (Doblado et al., 2005; Oboh and Akindahunsi, 2004; Oboh, 2006a, b). Cyclophosphamide (CP) is an alkylating agent and the most commonly used anticancer and immunosuppressant drug. It is used for the treatment of chronic and acute leukemia's, multiple myeloma, lymphomas and rheumatic arthritis and in the preparation for bone marrow transplantation (Dollery, 1999). The important factor for the therapeutic and the toxic effects of cyclophosphamide is the requirement of metabolic activation by the

ISSN: 0976-7126

hepatic microsomal cytochrome P450 mixed functional oxidase system (Ludeman, 1999). Phosphoramide mustard and acrolein are the two active metabolites of cyclophosphamide (Ludeman, 1999). Cyclophosphamide's antineoplastic effects are associated with the phosphoramide mustard, while acrolein is linked with its toxic side effects (Kern and Kehrer, 2002). Acrolein interferes with the tissue antioxidant defense system, produces highly reactive oxygen free radicals and is mutagenic to mammalian cells (Arumugam et al., 1997). However, recent reports revealed that intraperitoneal administration of cyclophosphamide would cause oxidative stress in brain (Bhatia et al., 2006). The plant *Garcinia Indica* belongs to family *Clusiaceae* popularly known as Vrikshamala in Hindi, Kokum in English. It is native to India, from the Western Ghats region of India, along the western coast. It is found in forest lands, riversides, and wasteland, and also gets cultivated on a small scale. The plant has been used in Hindu medicines from very early times. In ayurveda the plant is considered beneficial for the Flatulence, oedema, chronic alcoholism, cordil, digestive power, quenching thirst, mouth disease, substitute for daadima (Punica granatum), Carminative, astringent, Anticorbutic, healing ulceration, dysentery. The main constituent present in the fruit are especially from its rind, are rich in polyisoprenylated benzophenone derivatives such as garcinol and its colorless isomer, isogarcinol. The rind also contains hydroxyl citric acid (HCA), hydroxycitric acid lactones, citric acid and oxalic acid. The fruit also contains other compounds including malic acid, polyphenols, carbohydrates, anthocyanin, pigments and ascorbic acid. Garcinol shows strong antioxidant activity since it contains both phenolic hydroxyl groups as well as a β-diketone moiety, and in this respect it resembles with the well known antioxidant of plant origin, viz. Curcumin[7].This study seeks to assess the distribution of phenolic and antioxidants in *Garcinia Indica* fruit extract, and their ability to prevent cyclophosphamide-induced oxidative stress in the brain (Kokate et.al., 1999)

Material and Methods

Sample collection

Fruit were collected from the Pinnacle Biomedical Research Institute Bharat Scaud Guide Campus Shyamla Hills Bhopal. The authentication of the *Garcinia Indica* fruit was done in the Sarojni Naidu Govt. Girls P.G. College, Shivaji nagar, Bhopal, M.P. All the chemicals used were of analytical grade.

Sample preparation

The fresh fruit were separated from matured fruits, shade dried, broken into small pieces and powered coarsely. About 500 gm of air dried powdered material was extracted with 99.9% of methanol in a soxhlet extractor for 7 days. The extract was concentrated to dryness under reduced pressure and controlled temperature (20-25^{o} C) using rotary evaporator. The methanolic extract yielded a brown sticky mass weighing 9.2g and extractive value was found to be 3.326% w/w. the extract was used directly for total phenol and flavonoid content and also for assessment of antioxidant capacity through various chemical assays (Kokate et.al., 1999 and Mehta, 1999).

Estimation of total phenolic content

The total phenol content of the extracts was determined using the method reported by (Singleton et.al., 1999). Appropriate dilutions of the extracts were oxidized with 2.5 ml 10% Folin–Ciocalteau's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45^{0} C and the absorbance was measured at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

Evaluation of *in-vitro* antioxidant activity

DPPH radical scavenging assay

The free-radical scavenging ability of the extracts against 1,1-diphenyl-2 picryl hydrazyl free radical was evaluated as described by (Gyamfi et.al., 1999). Briefly, appropriate dilution of the extracts (1 ml) was mixed with 1 ml, 0.4 mM methanolic solution containing DPPH radicals; the mixture was left in the dark for 30 min and the absorbance was taken at 516 nm. The DPPH free-radical scavenging ability was subsequently calculated.

Reducing power activity

The reducing property of the extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by (Oyaizu et.al., 1986). In total, 2.5 ml aliquot was mixed with 2.5 ml 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50^{0} C for 20 min and then 2.5 ml 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. In total, 5 ml of the supernatant was mixed with

an equal volume of water and 1 ml 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant property (FRAP) was subsequently calculated as ascorbic acid and gallic acid equivalent, respectively.

Bioassay

The ability of the fruit of the *Garcinia Indica* to prevent cyclophosphamide induced oxidative stress in rat's brain was evaluated using the method reported by (Bhatia et.al., 2006). Wistar strain of albino rats weighing 180–235 g were collected from Pinnacle Biomedical Research Institute Bhopal (M.P.) and acclimatized for 2 weeks, during which period they were maintained *ad libitum* on commercial diet. The rats were subsequently divided into four treatment groups. Animals in groups 1 and 2 were feed the basal diet while Animals in groups 3 were feed the basal diet with *Garcinia Indica* fruit extract-I (250mg/kg of body weight) respectively, and those in groups 4 were feed the basal diet with *Garcinia Indica* fruit extract-II (500mg/kg of body weight). The experiment last for 14 days. The rats in groups 2–4 were injected intraperitoneally with cyclophosphamide (75mg/kg of body weight) 24 h before the termination of the experiment, while group 1 served as the control. The malondialdehyde content of the brain was determined as described earlier, and serum activities of glutamate oxaloacetate transferase (SGOT), glutamate pyruvate transferase (SGPT), alkaline phosphatase (ALP) and total bilirubin were determined using diagnostic kits.

Data analysis

The results of the three replicates were pooled and expressed as mean and standard error (S.E.). Student t-test, one-way analysis of variance and the least significance difference were carried out (Zar, 1984). Significance was accepted at $P \geq 0.05$.

Results and Conclusion

The phenolic distribution in the *Garcinia Indica* fruits as presented in Table1 that the total phenol content of the *Garcinia Indica* fruits (2.94%) was significantly higher (P>0.05). The antioxidant indices of phenolic extracts of the *Garcinia Indica* fruits typified by the ferric reducing antioxidant property and DPPH scavenging ability presented in Table1 that there was a dose dependent response in the ability of phenolics to reduce Fe3+ to Fe2+ (Table1) and in the ability to scavenge DPPH free radicals. The ferric reducing antioxidant property of the *Garcinia Indica* as ascorbic acid equivalent was higher than that of the gallic acid equivalent. Polyphenols from the fruit pulp (gallic acid equivalent–51.45; Ascorbic acid equivalent–88.77) had a significantly higher (P>0.05).

In a bid to assess the protective effects of the *Garcinia indica in vivo*, Wistar strain albino rats were placed on diets with varying dosing levels of *Garcinia indica* fruit extract (250 mg/kg and 500 mg/kg BW) and did not show significant difference (P>0.05). The intraperitoneal administration of a single dose of cyclophosphamide (75 mg/kg BW) 24 h prior to the termination of the experiment caused a significant increase in the MDA content of the rat brain. However, rats that were dosing with *Garcinia indica* fruit extract showed a significant decrease ($P<0.05$) in brain MDA content (Table 2).

Furthermore, a significant increase ($P<0.05$) in SGOT, SGPT, ALP and total bilirubin levels following cyclophosphamide administration was observed (Table 3); there was also a significant decrease in the marker enzymes and metabolite levels of rats dose with *Garcinia indica* fruit extract, indicating a protection of the hepatocytes. Basal rats not dose with *Garcinia indica* fruit extract and not injected with cyclophosphamide. Control rats not dose with *Garcinia indica* fruit extract and injected with cyclophosphamide.

Diet has been known to play an important role in the prevention of certain diseases. Recent findings have linked diet to protecting the body against the exacerbating effects of unchecked ROS generation resulting in oxidative stress. Garcinia indica is an important fruit in western ghat regions india (Rao et al., 1988). It is an excellent source of natural phytonutrients such as vitamins C and E, polyphenols, Anthocyanins (Cyanidin-3-sambubioside, Cyanidin-3-glucoside), Fatty Acids (palmitic, stearic, oleic, linoleic acid), Hydroxycitric acid (HCA), Garcinol (Rao et al., 1988).which have been confirmed by epidemiological studies to reduce the risk of cancer and gastrointestinal diseases when taken in adequate amounts (Mishra et al.,2008).

The phenolic content of the Garcinia indica is found to be higher in whole fruit (Sengar et al., 2011). Polyphenols are considered to be strong antioxidants due to the redox properties of their hydroxyl groups (Materska and Perucka, 2005). Natural polyphenols are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce a-tocopherol radicals and inhibit oxidases (Oboh, 2006a). Reducing power can bean ovel antioxidation defense mechanism; the two mechanisms that are available to affect this reducing power are by electron transfer and hydrogen atom transfer (Dasmalchi et al., 2007). The reducing power of the extracts assessed based on their ability to reduce Fe (III) to Fe (II) show phenolic

extracts from Garcinia indica fruits as being able to reduce Fe (III) to Fe (II).

The decrease in MDA levels signifies an inhibition of peroxidation of membrane lipids at the in vitro level and this correlates with observations made by Oboh and Rocha (2007) on the ability of phenolic extracts of C. annum var. aviculare and Capsicum Chinese Habanero to prevent lipid peroxidation in vitro. Furthermore, the agreement in the phenolic distribution and reducing power and inhibition of lipid peroxidation suggests that the reduction of Fe (III), scavenging of free radicals and protection of membrane lipids may be mechanisms involved in the antioxidant capability of C. fruit scens var. abbreviatum.

The increase in brain MDA content following intraperitoneal administration of cyclophosphamide agrees with earlier report by Bhatia et al. (2006). The administration of an intermittent massive dosage of cyclophosphamide has been found to be advantageous in chemotherapy (Mythili et al., 2004). The cellular mechanism of its toxicity (i.e. cyclophosphamide) is mediated by an increase in the free radicals through intracellular phosphamide mustard and acrolein, the principal alkylating metabolite of cyclophosphamide (Senthil kumar et al., 2006). Acrolein interferes with the tissue antioxidant defense system, produces highly reactive oxygen free radicals and is mutagenic to mammalian cells (Senthil kumar et al., 2006).

Free radical induced lipid peroxidation has been suggested to alter the membrane structure and function, causing cellular abnormalities such as mutations and cell death (Senthil kumar et al., 2006). Hence, an increase in free-radical production mediated by cyclophosphamide metabolites, which in turn stimulated lipid peroxidation, leads to an increase in MDA production in the brain cells of rats. Coupled with this is the fact that the brain has limited access to the bulk of antioxidants produced by the body; hence neurons are the first cells affected by a shortage of antioxidants and are the most susceptible to oxidative stress (Oboh and Rocha, 2007).

The higher dose of Garcinia indica fruit extract (500mg/kg BW) resulted in the MDA levels being maintained to near normal status. The protective ability of the Garcinia indica fruit extract could be attributed to its high phenolic content with antioxidant properties. The findings of Sengar et al. (2011) have shown the ability of phenolic extracts of Garcinia indica fruit to prevent lipid peroxidation in vivo. It is also worth noting the inhibitory effect of Garcinia indica fruit dose on lipid peroxidation, showed a higher ($P<0.05$) inhibitory effect on MDA production in rat brain tissue. However, it is worth noting that the results of the protective effect to f the Garcinia indica fruit dose agrees with the phenolic distribution, antioxidant activities, and inhibition of lipid peroxidation in brain homogenates in vivo. This higher antioxidant and neuro protective effect of the Garcinia indica fruit (in vitro and in vivo) could be attributed to its high phenolic content. These findings agree with many earlier reports where a correlation was established between phenolic content and antioxidant properties of some plant foods (Sun et al., 2002; Chu et al., 2002; Oboh and Akindahunsi, 2004; Oboh et al., 2007).

Also, in the present study, cyclophosphamide administration was observed to significantly increase ($P<0.05$) SGOT, SGPT, ALP and total bilirubin levels in the blood serum. Increased activities of these enzymes in the serum are well known diagnostic indicators of liver injury. SGPT and SGOT are enzymes located in the liver cells and leak out, making their way into general circulation as liver cells are damaged (Oboh, 2006b). In acute liver injury such as acute viral hepatitis, SGPT and SGOT activities may be elevated very high, sometimes above 1000UI/L (Oboh, 2006b). Findings by Oboh and Akindahunsi (2005) have indicated that an increase in the serum levels of both transaminases indicates a possible damage to the liver.

The increased levels of these enzymes and metabolites could be because of acrolein's interference with the body antioxidant system. Abreach in the antioxidant defense system would result in proliferative production of ROS, which would in turn attack hepatocyte membrane disrupting its structure and function, the result of which is a leakage of these enzymes into the blood circulation. Similar studies by Mythili et al. (2004), Senthil kumar et al. (2006) and Sulkowska et al. (2002) showed that intraperitoneal injection of cyclophosphamide resulted in decrease in the activity of antioxidant enzymes and an increase in serum marker enzymes and metabolites for liver function.

Protective effect of the Garcinia indica fruit dose could have resulted from phytochemicals in the Garcinia indica fruit neutralizing free radicals generated by cyclophosphamide metabolites, thus protecting the cell membrane. This is in keeping with studies by Oboh and Rocha (2007a) that polyphenols in Garcinia indica fruit inhibit lipid peroxidation in hepatocytes. Also, the Garcinia indica fruit dose showed greater hepatoprotective ability. This is consistent with the earlier results of the in vitro and in vivo studies.

Acknowledgements

The authors would like to express their sincere thanks to Dr. Firoz Ahmed, Pharmacy Discipline, Khulna University for his active co-operation and important support during the experiments. The authors are also thankful to Prof. M.S.K. Choudhury, Ethnopharmacology Laboratory, Department of Pharmacy, Jahangirnagar University for providing laboratory facilities to conduct a part of the research work.

References

1. Shulman R G, Rothman D L, Behar K L, Hyder F. Energetic basis of brain activity implications for neuro imaging. Trends Neuro sci 2004; 27:489–95.
2. Perry S W, Norman J P, Litzburg A, Gelbard H A. Antioxidants are required during the early critical period, but not later, for neuronal survival. J Neuro sci Res 2004;78:485–92.
3. Doblado R, Zielinski H, Piskula M, Kozlowska H, Munoz R, Fria¨a s J,et al. Effect of processing on the antioxidant vitamins and antioxidant capacity of Vigna sinensis var. carilla. J Agric Food Chem 2005; 53:1215–22.
4. Oboh G, Akindahunsi A A. Nutritional and toxicological evaluation of Saccharomyces cerevisae fermented cassava flour. J Food Compos Anal 2005; 18:731–8.
5. Oboh G. Antioxidant properties of some commonly consumed and underutilized tropical legumes. Eur Food Res Technol 2006a; 244:61–5.
6. Oboh G. Hepatoprotective property of ethanolic and aqueous extracts of fluted pumpkin (Telfaria occidentalis) leaves against garlic-induced oxidative stress. J Med Food 2006b; 8:560–3.
7. Dollery C. Cyclophosphamide. In:Dollery C, editor. Therapeutic drugs. Edinburg: Churchill Livingstone; 1999. p. 349–53.
8. Ludeman S M. The chemistry of the metabolites of cyclophosphamide. Curr Pharm Des 1999; 5:627–43.
9. Kern J C, Kehrer J P. Acrolein induced cell death:acaspase influenced decision between apoptosis and oncosis/necrosis. Chem Biol Interact 2002; 139:79–95.
10. Arumugam N, Sivakumar V, Thanislass J, Devaraj H. Effects of acrolein on rat liver antioxidant defense system. Indian J Exp Biol 1997; 35:1373–4.
11. Bhatia A L, Manda K, Patni S, Sharma A L. Prophylactic action of linseed (Linum usitatissimum) oil against cyclophosphamide induced oxidative stress on mouse brain. J Med Food 2006; 9:21–264.
12. Kokate CK, Purohit AP, Gokhle SB. Textbook of pharmacognosy; 1999, (XII): 290-295.
13. Mehta RM, Textbook of pharmaceutics; 1999, (II): 146-14.
14. Singleton VL, Orthofor R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocaltau reagent. Meth- ods Enzymol 1999; 299:152–78.
15. Gyamfi MA, Yonamine M, Aniya Y. Free-radical scavenging action of medicinal herbs from Ghana: Thonningia sanguinea on experimentally-induced liver injuries. Genet Pharmacol 1999; 32:661–7.
16. Oyaizu M. Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. Japan J Nutr1986; 44:307–15.
17. Belle N A V, Dalmolin G D, Fonini G, Rubim M A, Rocha J B T. Polyamines reduces lipid peroxidation induced by different pro-oxidant agents. Brain Res 2004; 1008:245–51.
18. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reactions. Anal Biochem 1979; 95:351–8.
19. Zar J H. Biostatistical analysis. Upper Saddle River, N J Prentice-Hall; 1984.

ISSN: 0976-7126

Table 1: Total phenol, reducing power and DPPH free-radical scavenging ability of *Garcinia indica* Fruit Extract

Total phenol (%)	Reducing power (mmol/g)		DPPH Free-radical scavenging activity (%)	
	Gallic acid equiv.	Ascorbic acid equiv.	0.5mg/ml	1.0 mg/ml
2.94±.03	51.45±0.8	88.77±1.4	44.87±0.4	70.87±1.5

Table 2: MDA content of the rat brain

TREATMENT GROUP	LPO (MEAN±SEM)	GSH (MEAN±SEM)	CATALASE (MEAN±SEM)	SOD (MEAN±SEM)
Vehicle (CMC)	0.697±0.048	4.33±0.044	338.4±4.19	85.59±2.635
Control(CYP)	4.25±0.038	0.87±0.096	199.8±3.95	49.54±3.227
Extract 250 mg/kg + CYP	1.62±0.048	1.95±0.075	270±3.62	65.45±2.708
Extract 500 mg/kg + CYP	1.95±0.052	2.15±0.082	240±3.72	60.42±2.815

Table 3: SGOT, SGPT, ALP and Total Billirubin levels in rat serum

TREATMENT GROUP	SGOT (MEAN±SEM)	SGPT (MEAN±SEM)	ALP (MEAN±SEM)	BILIRUBIN (MEAN±SEM)
Vehicle (CMC)	68.34±2.46	55.1±2.53	29.84±1.55	0.483±0.0032
Control (CYP)	255.1±5.5	219.6±4.85	137.6±4.37	3.907±0.062
Extract 250 mg/kg + CYP	130.6±3.42	103.4±2.6	80.45±2.59	1.95±0.0396
Extract 500 mg/kg + CYP	159.5±4.12	120.2±3.4	92.25±3.32	2.12±0.042