

Research Article

Myeloprotective potential of *Malus domestica* (Apple) in Carboplatin induced myelosuppression in Swiss albino rats-A way to prevent Dengue and similar viral diseases

Maaz Ahmad¹, Mussab Ahmad², Tehreem Munir³

¹Professor of Community Medicine, Rashid Latif Medical College, Lahore; Former Dean Institute of Public Health, Lahore/ Former Dean Faculty of Preventive Medicine, King Edward Medical University, Lahore; ²Medical Officer, Mayo Hospital, Lahore; ³Medical Officer, Mayo Hospital, Lahore

Abstract |

Background: It is a well-recognized clinical observation that there is bone marrow suppression in viral diseases affecting humans and also as a result of chemotherapy. Many plants and fruits are claimed to possess the quality of myeloprotection. If so, they might be used for prevention of viral diseases.

Objective: To evaluate the potential of epicarp extract of *Malus domestica* to prevent myelosuppression induced by carboplatin.

Materials and Methods: Epicarp extract of *Malus domestica* (M.domestica) was prepared through anaerobic sublimation and ethanolic-aqueous filtration. Two groups of Swiss albino rats were taken. One group was given Carboplatin (an anticancer agent) in the dose of 125 mg/kg to induce bone marrow suppression whereas second group was given carboplatin in the same dose along with 100mg/Kg epicarp extract of M. domestica. Blood examination was carried out on day 1 and after 7 days to see the effects of the extract. Results were analyzed through SPSS version 21.

Results: Myelosuppression was observed in both Groups. On average there was more myelosuppression in the Carboplatin group alone marked by 87.53% reduction in platelets count, 66.28% reduction in RBC and 67.11% reduction in WBC count as compared with much less myelosuppression in trial group evidenced by 22.83% ,6.61% and 18.79% reduction in platelets, RBC and WBC count respectively. It was evident that there was significant myeloprotection in the trial group ($p < 0.001$).

Conclusions: Ethanolic-aqueous epicarp extract of M.domestica seemed to possess myeloprotective properties.

Received | 07-06-2017: **Accepted** | 15-12-2018

Corresponding Author | Prof. Maaz Ahmad, Professor of Community Medicine, Rashid Latif Medical College, Lahore, Former Dean Institute of Public Health, Lahore. **Email:** profmaaz@gmail.com

Keywords | M.domestica, Apple peel, Ethanolic-aqueous epicarp extract, Myelosuppression, Dengue.

Introduction

Every year millions of people suffer from communicable diseases and graph of mortality goes high. Why do these people victimized by so many horrifying micro-organisms? Sometimes a new variant attacks the innocent population and shakes the

health system. Vaccine is not available against many of them and other preventive measures get delayed due to some unavoidable issues. In the near past several viral infections like Dengue, Congo, Zika and Chikungunya have attacked human being. Dengue fever is one of the emerging infections in Pakistan. Dengue fever is spreading due to new strains, resis-

tance to insecticides, travelling and urbanization.¹

As there was no effective vaccine against dengue and other new emerging viral infections, major focus remained to control the vector and personal protection. There had been use of some natural substances regarding treatment and prevention but yet to be scientifically proved.

As far as treatment was concerned, many attempts had already been made using natural substances here and other dengue endemic countries. Papaya leaves and apple fruit were quite popular for early recovery in dengue patients. There was much supportive evidence present in favor of papaya leaves in the literature as elaborated in different studies. In a study prevention of thrombocytopenia induced by carboplatin was revealed with the help of papaya leaf juice.² In another study aqueous extract of *Carica papaya* increased the platelets count in cyclophosphamide induced thrombocytopenic rats.³ Similar results were obtained in a study which revealed increase in count of platelets when *Carica papaya* leaf extract was given to dengue patients.⁴ As far as apple is concerned every body is aware about the saying that having an apple daily keeps the doctor away.⁵ During dengue epidemics fresh apple juice mixed with lemon juice has been used by dengue patients and found effective.⁶ Attempts were made to devise a nutritional formula to increase platelet count in dengue patients.⁷ As we know, apple is full of powerful antioxidant and phytochemicals like catechin, quercetin, chlorogenic acid and phloridzine. Both apple flesh and peel contain compounds like phloretin glycosides, phloridzin, and chlorogenic acid. However, other flavonoids and polyphenolic compounds (e.g., procyanidins etc) which are powerful antioxidant are found exclusively more in the apple peel as compared to flesh.^{8,9,10} Apple peel's protective effects are quite pronounced regarding cardiovascular diseases, diabetes, cancers and asthma. Apple peel extract was found very effective protector against liver, breast and colon cancer due to its strong antiproliferative property.⁹ Studies conducted revealed its strong antioxidant property by inhibiting cancer cell proliferation and decreasing lipid oxidation.¹¹ Polyphenols in apples' colored skin protected them from bacteria, viruses, fungi, and the damaging effects of UV-B radiation.¹²

Apple peels are naturally full of nutrients and fiber. It

is a fact that there are more than 2–6 times nutrients and insoluble fiber in the apple peel as compared with the flesh of apples. It is rightly said that consuming a skin-on apple per day keeps spare pounds away.¹³ There are so many health benefits in apple peel. Apple peel enhances power of concentration and improves memory by protecting brain cells. Respiratory problems are resolved after regular use of apple peel. It prevents cataract. It stops formation of gall stones. It may be used in management of anemia.¹⁴ It helps in bone strengthening. Overall body immune system is augmented due to its antioxidant property, flavonoids and phytochemicals. It is effective cholesterol lowering agent.¹⁵ In the past, research work had been done on apple peel and beneficial effects of apple peel were observed as was evident in one study in which consumption of dried apple peel powder was found to be associated with improved joint function and improved serum antioxidant protection status.¹⁶

A study was conducted to define the polyphenols extracted from dried apple peels (DAPP) and determine their antioxidant and anti-inflammatory role. Findings provided evidence of the capacity of DAPP to reduce oxidative stress and inflammation which are known pivotal processes involved in inflammation.¹⁷ In another study apple peel extract prevented indomethacin-induced damage in Caco-2 cells through preventing mitochondrial complex I inhibition.¹⁸

Though there were reports about the usefulness of intake of apple juice in minimizing the agony of dengue disease but less scientific evidence was available specially about apple peel.

As we know that all blood cells develop from a small subset of the same progenitor cells in the bone marrow through a process called hematopoiesis. Hematopoietic stem cells give rise to red and white blood cells and platelets

Mechanism of dengue infection is not yet fully understood but a well-recognized clinical observation documented in virus-induced human diseases is that there is marked myelosuppression similar to radiation therapy or anticancer chemotherapy.^{19,20,21} Similar case is of dengue where there is evidence that during the acute phase of the disease dengue virus can induce bone marrow hypoplasia.²² It was found in an early investigation of dengue cases in Southeast Asia that

the bone marrow mass was at its lowest level just prior to the onset of fever.²³

It was postulated that most probably any thing which could check this bone marrow suppression in the initial phase of dengue disease might be used to prevent the onset of disease. So an animal study was designed to check the myeloprotective potential of the epicarp extract of *M.domestica* through oral administration to attenuate carboplatin-induced bone marrow suppression in Swiss albino rats .

Methods

Ripe fruits of *M.domestica* (Apple) were freshly procured from a local market. The non-edible epicarp was separated from the edible fruit, and dried under shade. 200g of the dried epicarp powder was undergone anaerobically sublimation followed by ethanolic-aqueous extraction for a period of 6 weeks (microprevention technique) to get extract. The resultant extract was reconstituted to make 1 ppm solution and stored in the refrigerator until needed.

A randomized control trial was conducted to evaluate the myeloprotective potential of epicarp extract of *M.domestica* in carboplatin induced myelosuppression. Twenty male Swiss albino rats aged seven to eight weeks, weighing 25-35 g were purchased and housed in the animal house of Post Graduate Medical Institute, Lahore. They were kept together for two weeks to acclimatize and were fed with commercially available rodent chow and had access to take feed and

drink water ad libitum. Two groups of 10 rats each after random selection, labeled 1 and 2 were made. Certain antineoplastic drugs are directly cytotoxic to myeloid stem cells or their progenitors where-as others affect hematopoiesis indirectly. Carboplatin, an antineoplastic drug, was selected to induce myelosuppression in this study. Group 1 rats were administered 125 mg/kg of carboplatin as single intraperitoneal injection to induce bone marrow suppression on day zero, whereas Group 2 rats were administered carboplatin along with 100mg/kg epicarp extract of *M.domestica* orally for seven days.² Blood samples (0.5 ml) were collected from each animal through cardiac puncture under light anesthesia on day zero and seven and sent for hematological analysis. RBC, WBC and platelet counts were used as parameters for comparison. Data was analyzed using SPSS version 21. Independent sample 't' test was carried out to find out the difference between both groups (Table 3). Significant differences were found at 95% confidence level, with the $P < 0.05$ being considered as significant.

Results

Rats were divided in 2 groups. Carboplatin was given to Group 1 rats as per SOP defined earlier. RBC, WBC and platelet counts were recorded on Day 0 and Day 7. There was observed expected suppression in these blood cells (Table 1)

Table 1: Table.1. Effect of Carboplatin on Blood Cells given to Group 1 Rats

	Platelet Count(10^9 /L)		Percent Fall(%)	RBC Count (10^{12} /L)		Percent Fall(%)	WBC Count (10^9 /L.)		Percent Fall(%)	
	Day 0	Day 7		Day 0	Day 7		Day 0	Day 7		
1	643	60	90.67	6.53	3.53	45.94	4.76	1.87	60.71	
2	621	75	87.92	7.12	2.63	63.06	4.87	1.56	67.97	
3	432	85	80.32	6.54	2.02	69.11	6.12	2.55	58.33	
4	494	91	81.58	6.58	2.25	65.81	6.76	2.13	68.49	
5	659	85	87.10	7.19	2.76	61.61	5.98	1.02	82.94	
6	793	64	91.93	6.17	1.19	80.71	7.85	2.76	64.84	
7	640	75	88.28	6.98	1.96	71.92	7.45	2.97	60.13	
8	739	56	92.42	6.04	1.99	67.05	7.43	2.61	64.87	
9	629	83	86.80	6.52	2.32	64.42	8.80	2.22	74.77	
10	709	83	88.29	6.99	1.87	73.25	6.97	2.23	68.01	
Average Myelosuppression(%)			87.53				66.28			

Table revealed that there was marked myelosuppression in all rats ($P < 0.001$) in Group 1.

Group 2 rats were given Carboplatin and extract of *M. domestica* in the already specified doses simultaneously. RBC, WBC and platelet counts were recorded on Day 0 and Day 7.

Regarding blood cell count there was no significant difference on zero day in the two groups of rats ($p > 0.05$). However after 7 days there was significantly less myelosuppression in group 2 as compared with group 1. (Table 2)

Table 2: Effect of simultaneously given Carboplatin and Epicarp extract of *M. Domestica* on Blood Cells to Group 2 Rats.

	Platelet Count(10^9 /L)			RBC Count(10^{12} /L)			WBC Count(10^9 /L)		
	Day 0	Day 7	Percent Fall(%)	Day 0	Day 7	Percent Fall(%)	Day 0	Day 7	Percent Fall(%)
1	756	605	19.97	7.52	6.53	13.16	6.73	5.04	25.11
2	665	528	20.60	7.83	6.63	15.33	8.65	6.56	24.16
3	592	222	62.50	6.91	7.02	-1.59	5.41	4.55	15.90
4	534	421	21.16	6.84	6.24	8.77	7.74	6.13	20.80
5	659	502	23.82	7.69	7.44	3.25	4.36	3.87	11.24
6	678	532	21.53	6.77	6.54	3.40	7.90	5.76	27.09
7	590	478	18.98	6.83	6.23	8.78	6.12	5.67	7.35
8	610	463	24.10	7.12	6.97	2.11	6.34	5.12	19.24
9	679	589	13.25	6.87	6.43	6.40	7.75	6.64	14.32
10	589	575	2.38	7.23	6.76	6.50	6.43	4.97	22.71
Average Myelosuppression(%)			22.83			6.61			18.79

Significant difference was found between the two groups at the end of trial period and epicarp extract of

M. domestica was found significantly myeloprotective ($p < 0.001$). See Table 3.

Table 3: Comparison between Group 1 and Group 2 at the end of Trial. (Day 7)

Sr. No.	Percent Fall(%) in Platelet Count Group 1	Percent Fall(%) in Platelet Count Group 2	Significance Independent sample 't' test	Percent Fall(%) In RBC count Group 1	Percent Fall(%) In RBC count Group 2	Significance Independent sample 't' test	Percent Fall(%) in WBC Count Group 1	Percent Fall(%) in WBC Count Group 2	Significance. Independent sample 't' test
1	90.67	19.97	$P < 0.00$	45.94	13.16	$P < 0.00$	60.71	25.11	$P < 0.00$
2	87.92	20.60		63.06	15.33		67.97	24.16	
3	80.32	62.50		69.11	-1.59		58.33	15.90	
4	81.58	21.16		65.81	8.77		68.49	20.80	
5	87.10	23.82		61.61	3.25		82.94	11.24	
6	91.93	21.53		80.71	3.40		64.84	27.09	
7	88.28	18.98		71.92	8.78		60.13	7.35	
8	92.42	24.10		67.05	2.11		64.87	19.24	
9	86.80	13.25		64.42	6.40		74.77	14.32	
10	88.29	2.38		73.25	6.50		68.01	22.71	
Myeloprotection(%)			Significant	Myeloprotection(%)		Significant	Myeloprotection(%)		Significant

Discussion

This study was designed to evaluate the effect of apple peel extract on carboplatin induced myelosuppression in mice as animal model.

In the past, researches have been conducted on health

benefits of apple and apple peel.^{2,3} Generally viral diseases specially dengue produces acute myelosuppression in very early phase of disease^{19,20} and it was postulated that if this myelosuppression gets checked in time might lead to no onset of disease.^{21,22,23} The current study is unique in this aspect that for the first

time apple peel was investigated to check carboplatin induced myelosuppression in rats so opening the doors for further research for human health regarding other parts of *M. domestica* like pulp, seeds, flowers, leaves or roots.

The idea of role of apple peel emerged when dengue hit Lahore, Pakistan in 2011 in epidemic fashion and people tried many fruits like Papaya leaves, apple juice, different edible seeds and cereals to reduce the agony of disease.⁴ Good results were achieved in some of the cases giving the impression of protective and preventive role of these fruits in dengue but most of them could not be properly documented.⁵ Many researchers here and other dengue endemic countries did a hard work to evaluate papaya and apple regarding treatment of dengue disease and in some studies the role of papaya extract in the inhibition of thrombocytopenia in dengue disease was highlighted.^{2,3,4} In Kuala Lumpur, Malaysia, Prof. Dr. Ananthan Krishnan formulated a product with the highly active micro-nutrients found in edible seeds, cereals and fruits like papaya as well as vitamins to increase blood platelets in dengue disease.⁷ It is interesting to note that from studies conducted using papaya juice encouraged to use other things like apple peel to determine its efficacy in checking myelosuppression.⁴ In the literature much has been said about the health benefits of apple peel which is not given due importance in our daily life as we do not eat it rather discard. Apple peel is not an ordinary thing. There are so many health benefits of apple peel. Apple peel as we know is rich in nutrients and by owing such wealth, it enhances brain functions.^{11,12} It is also good for Alzheimer's and Parkinson's disease.¹² It also relieves breathing problem.^{11,14} It is a gift of God for eye health and delays the onset of cataract.¹³ It also prevents formation and accumulation of stones in the gall bladder.¹⁴ As it is rich in folic acid and iron, so it helps anaemic individuals specially pregnant mothers.¹² Calcium present in the apple peel helps bone formation and bone repair.¹⁴ It lowers cholesterol.¹³ Triterpenoids present in the apple peel have cancer fighting properties and help to protect from cancer such as liver, breast and colon cancer.^{11,12,15}

But the most important aspect of apple peel is that it is rich in antioxidants which fight against oxidative stress induced by various viruses, chemotherapeutic agents and radiation.^{11,12,16} This antioxidant content

along with flavonoids and phytochemicals, body immune system is strengthened to make the body capable to cope with various diseases.^{17,18}

Though some studies have been conducted in the past to evaluate the efficacy of papaya fruit juice³ and papaya leaves^{2,4} in preventing thrombocytopenia and increasing the platelet count in animals but no study could be traced out focusing the inhibition of thrombocytopenia induced by carboplatin by ethanolic-aqueous extract of apple peel so far. Studies available about apple peel extract are mostly highlighting its antiproliferative activity preventing different types of cancers. Role of apple peel may not be underestimated as it contains so powerful antioxidants to combat with oxidative stress induced by fatal viruses.

In the current study, we tested the hypothesis that oral administration of the ethanolic aqueous apple peel extract prevented myelosuppression induced by carboplatin, an anticancer agent. The results clearly revealed that myelosuppression was observed in both groups but Group B rats receiving Carboplatin and herbal extract simultaneously showed significant maintenance ($P < 0.001$) in cellular count when compared with the myelosuppressed rats (Group A), indicating that this extract might possess good myeloprotective properties.

So if more research is focused on this aspect, there is strong possibility that we might be able to discover a safe, economical and effective way to check the initiation of myelosuppression in the beginning of dengue and other viral diseases.

This study may be considered as pilot study and needs many more to support it. There is dire need to probe other plants, fruits and vegetable possessing antioxidant properties to fight against various diseases.

Conclusion

This study has demonstrated that oral administration of the ethanolic aqueous apple peel extract protected bone marrow in spite of myelosuppression effect of carboplatin in Swiss albino rats. These observed changes in the hematological parameters show the myeloprotective ability of the extract. Characterization of the epicarp extracts and identification of the active components necessitate further research. However the results of this study apparently support

the general observation of using *M.domestica* for the prevention and treatment of dengue fever and its complications in general community. In fact this study reveals first time the preventive role of epicarp extract of *M.domestica* which opens a new horizon for prevention of viral diseases not having vaccines so far. This correlation needs further verification and is open for research. Further large-scale studies could establish the usefulness or ineffectiveness of this natural product in the treatment/prevention of dengue.

Ethical Approval: Given

Conflict of Interest: None

Funding Source: None

References

1. Evelyn N, Murray A, Quam MB, Smith AW. Epidemiology of dengue: past, present and future prospects. *Clin Epidemiol*. 2013; 5:299–309.
2. Tahir N, Zaheer Z, Kausar S. Prevention of fall in platelet count by *Carica papaya* leaf juice in carboplatin induced thrombocytopenia in mice. *Biomedica*. 2014 ;30(1):21-25.
3. Patil S, Shetty S, Bhide R. Evaluation of platelet augmentation activity of *Carica papaya* leaf aqueous extract in rats. *J Pharmacogn and Phytochem*. 2013;1(5):57-60
4. Gadhwal AK, Ankit BS, Chahar C. Effect of *Carica papaya* leaf extract capsule on platelet count in patients of dengue fever with thrombocytopenia. *J Assoc Physicians India*. 2016;64(6):22-26.
5. Davis MA, Bynum JP, Sirovich BE. Association between apple consumption and physician visits: appealing the conventional wisdom that an apple a day keeps the doctor away. *JAMA internal medicine*. 2015 ;175(5):777-83.
6. Sunidhi M., Kiran A, Dharti K.S. Prevention and control of dengue by diet therapy. *IJMR* 2017; 4(1):13-18.
7. Sarala N, Paknikar SS. Papaya Extract to Treat Dengue: A novel therapeutic option? *Ann Med Health Sci Res*. 2014;4(3):320–24.
8. Ravi K, Preshant A. Evaluation of antioxidant activity of apple peel and pulp extracts by using different solvents. *Chemical Science Transactions*. 2015; 4(3):723-27
9. Guabiraba R, Rydel B. Dengue virus infection: current concepts in immune mechanisms and lessons from murine models. *Immunology*. 2014;141(2):143-56.
10. Jelodarian S, Ebrahimabadi AH, Khalighi A, Batooli H. Evaluation of antioxidant activity of *Malus domestica* fruit extract from Kashan area. *Avicenna J Phytomed*. 2012; 2(3):139–45.
11. Zardo DM, Zielinski AA, Alberti A, Nogueira A. Phenolic Compounds and Antioxidant Capacity of Brazilian Apples. *Food and Nutrition Sciences*, 2015; 6(08):727-35.
12. Magdalena L, Tadeusz T, Bogusław B. Study of Antioxidant Activity of Biologically Active Compounds Isolated from Green Vegetables by Coupled Analytical Techniques. *Food Analytical Methods*. 2013;6(2):630–36.
13. Heeba GH, Mahmoud ME, El Hanafy AA. Anti-inflammatory potential of curcumin and quercetin in rats: Role of oxidative stress, heme oxygenase-1 and TNF- α . *Toxicol Ind Health*. 2014;30(6):551-60
14. Giomaro G, Karioti A, Bilia AR, Bucchini A, Giamperi L, Ricci D, et al. Polyphenols profile and antioxidant activity of skin and pulp of a rare apple from Marche region (Italy). *Chemistry Central J*. 2014 ;8(1):45.
15. Ravn-Haren G, Dragsted LO, Buch-Andersen T. Intake of whole apples or clear apple juice has contrasting effects on plasma lipids in healthy volunteers. *Eur J Nutr*. 2013 ;52(8):1875-89.
16. Jensen GS, Attridge VL, Benson KF, Beaman JL, Carter SG, Ager D. Consumption of dried apple peel powder increases joint function and range of motion. *J Med Food*. 2014 ;17(11):1204-13.
17. Denis MC, Desjardins Y, Furtos A. Prevention of oxidative stress, inflammation and mitochondrial dysfunction in the intestine by different cranberry phenolic fractions. *Clin Sci (Lond)*. 2015;128(3):197-212.
18. Lay MK, González PA, León MA. Advances in understanding respiratory syncytial virus infection in airway epithelial cells and consequential effects on the immune response. *Microbes Infect*. 2013;15(3):230-42.
19. Tsai JJ, Liu LT, Chang K, Wang SH, Hsiao HM, et al. The importance of hematopoietic progenitor cells in dengue. *Therapeutic Advances in Hematology*. 2012;3:59–71
20. Hottz ED, Oliveira MF, Nunes PC. Dengue induces platelet activation, mitochondrial dysfunction and cell death through mechanisms that involve DC-SIGN and caspases. *J Thromb Haemost*. 2013;11(5):951–62.
21. Sansanee N, Nattawat O, Hui-Mien H. Infection of bone marrow cells by dengue virus in vivo. *Exp Hematol*. 2012;40(3): 25059.