

The Journal of Phytopharmacology

(Pharmacognosy and phytomedicine Research)

Research Article

ISSN 2230-480X

JPHYTO 2016; 5(3): 112-116

May- June

© 2016, All rights reserved

G.M. Masud Parvez

Department of Pharmacy,
Varendra University, Rajshahi,
Bangladesh

Dr. Ashik Mosaddik

Department of Pharmacy, Rajshahi
University, Rajshahi, Bangladesh

Correspondence:

G.M. Masud Parvez

Department of Pharmacy,
Varendra University, Rajshahi,
Bangladesh

Email: masud.ph.ru[at]gmail.com

Evaluation of anticancer property of mango peel and flesh after formalin treatment

G.M. Masud Parvez, Ashik Mosaddik

ABSTRACT

Cancer is life threatening disease and the second leading cause of death. Various phenolic compounds obtained from plant sources such as fruit, bark, leaf and roots acts against cancer but when such fruits are treated with some chemicals such as formalin which itself a carcinogenic agent changes antioxidant potential of that fruit. To evaluate the change of anticancer potential fresh raw mangoes are collected and treated with formalin for 7 days and various parameters such as tumour cell growth inhibition, increase life span are measured. Various haematological parameters such as red blood cell, white blood cell and haemoglobin content is measured. It is found that normal mango peel contain higher anticancer property as compare to mango flesh. But when this mango peel is treated with formalin, its anticancer potential is decreases, not only in the peel but also in the flesh, which suggests the presence of formalin in mango flesh. From the study it is observed that normal mango peel (NP) causes significant inhibition of tumor cell growth (76.74%) and sufficiently increases life span (76.00%) at 100mg/kg daily dose whereas formalin treated peel (FP) causes (52.69%) tumour cell growth inhibition and increases lifespan (57.29%) at the same dose. On haematological study, normal mango peel increases red blood cell and Haemoglobin content and decreases white blood cell content but formalin treated sample does not make sufficient change. The anti-cancer activity shows a direct correlation with the dose

Keywords: Mango, formalin, anticancer, haematological.

INTRODUCTION

Research is now focusing on the search for new types of natural chemotherapeutic agent that is plant based medicines which are proving to be excellent sources of new compounds. A survey lists over 1400 genera of herbs that have a history of use in cancer treatments^[1]. Cancer continues to represent the one of the largest cause of mortality in the world and claims over 6 million lives each year^[2]. In 2007, cancer caused about 13% of all human deaths worldwide (7.9 million) and the rates are rising in the developing world^[3]. In 2008, approximately 12.7 million cancers were diagnosed (excluding non-melanoma skin cancers and other non-invasive cancers), and in 2010 nearly 7.98 million people died^[4]. In 2012, 8.2 million people died due to cancer throughout the world^[5]. Recent data suggests that 196,900 peoples are diagnosed of cancer and 78,000 deaths are occurred in Canada in 2015 due to cancer^[6], while in 2012, 186,400 new cases of cancer (excluding non-melanoma skin cancer) was diagnosed and 75,700 cancer deaths was occurred in Canada^[7]. In 2015 about 1,658,370 new cases of cancer have been identified and about 589,430 peoples are died in United States^[8]. In Australia, 130,470 new cases of cancer was diagnosed in 2015, with that number set to rise to 150,000 by 2020^[9].

Particular substances have been linked to specific types of cancer. Tobacco smoking is associated with many forms of cancer^[10] and causes 90% of lung cancer^[11]. In Western Europe 10% of cancers in males and 3% of cancers in females are attributed to alcohol^[12]. Cancer related to one's occupation is believed to represent between 2–20% of all cases^[13]. Physical inactivity is believed to contribute to cancer risk not only through its effect on body weight but also through negative effects on immune system and endocrine system^[14]. A high-salt diet is linked to gastric cancer, aflatoxin B1, a frequent food contaminate, with liver cancer, and betel nut chewing with oral cancer^[15]. Gastric cancer is more common in Japan due to its high-salt diet^[16] and colon cancer is more common in the United States. Bacterial infection may also increase the risk of cancer, as seen in *Helicobacter pylori* induced gastric carcinoma^[17]. Parasitic infections strongly associated with cancer include *Schistosoma haematobium* (squamous cell carcinoma of the bladder) and the liver flukes^[18]. Less than 0.3% of the population are carriers of a genetic mutation which has a large effect on cancer risk and this cause less than 3–10% of all cancer^[19]. Usually, physical carcinogens such as asbestos, fib ers, glass wool and rock wool also cause to develop cancer^[20].

From newspaper report it is found that mango, one of the most cultivated fruits in the world along with other fruits such as litchi, blackberry are regularly treated with formalin to keep them stay for long time. A survey in 26 markets in Dhaka city by environment protective agency "Save the Environment

Movement" (SEM) during June 1 to 10, 2013 found that around 94 percent of the mangoes and 100 percent blackberries and litchis are formalin-tainted [21]. A similar result is found in the following year, tests conducted by Poribesh Bachao Andolon (POBA) from June 1 and June 10, 2014 show that, all the black berries and 95 percent litchis sold in 35 city kitchen markets of Dhaka city contained formalin [22]. Literature reports suggests that there is sufficient study about anticancer activities of mango but there is no study about the anticancer potentials of mango after formalin treatment [23-26]. The objective of the present study is to find out the effects of formalin on anticancer property of mango.

MATERIALS AND METHODS

Collection of plant material

About 10 kg of raw mango was collected from the garden as pure state and it does not contain any foreign chemical. After collecting the mango were washed out in distilled water and shed dried. The mango was divided into two groups, one group is treated with formalin for 7 days and other group was kept as normal. The formalin solution was sprayed by the spray gun. Both groups of mangoes were peeled off and peels and flesh are oven dried at 55°C. The peels and flesh of each group are then ground into coarse powder. The samples are then extracted by ethanol under sonication bath and filtered. The filtrate was then concentrated with a rotary evaporator under reduced pressure at 50°C to obtain brownish mass of peels and reddish mass of flesh.

Transplantation of ascite tumour

Ascitic fluid was drawn out from different tumour bearing Swiss albino mice at the respective log-phases of tumour cells. A 3ml syringe filled with 20 gauge needle was used for this tumour cell aspiration. The freshly drawn fluid was diluted with normal saline (0.98% NaCl solution) and the tumour cells number was adjusted to approximately 2×10^6 cells / ml by counting the cell number with the help of a haemocytometer. The viability of tumour cells was observed by trypan blue dye (0.4%) exclusion assay. Cell sample showing above 90% viability were used for transplantation. Tumour suspension of 0.1 ml was injected intraperitoneally (i. p.) to each Swiss albino mouse. Strict aseptic condition was maintained throughout the transplantation process.

Preparation of stock solution

For therapeutic treatment, stock solution of bleomycin was made by using distilled water at the concentration of 0.075 mg/ml. Crude mango extract was dissolved in pure distilled water at the concentrations of 6.25 mg/ml and 12.5 mg/ml.

Determination of cell growth inhibition

To determine the cell growth inhibition, six groups of Swiss albino mice (6 in each group) weighing 20-25 gm were used. For therapeutic evaluation 13.6×10^4 EAC cells in every mouse were inoculated into each group of mice on day "0". Treatments were started after 24 hours of tumour inoculation and continued for five days. Group one to four received the test compound at the doses of 50 mg/kg (i. p.), and 100 mg/kg (i. p). Group five received bleomycin at the dose of 0.3 mg/kg (i. p) and group six was used as control. Mice in each group were sacrificed on day six and the total intra peritoneal tumour cells were harvested by normal saline (0.98%). Viable cells were first identified by using trypan blue and then counted by a haemocytometer. Total number of viable cells in every animal of the treated groups was compared with those of control (EAC treated only) group.

Determination of survival time and increase lifespan

For this determination, [27] method was followed. Tumour growth were monitored and host survival was recorded and expressed as mean survival time in days and percent increase of life span was calculated by using the following formulae:

$$\text{Mean survival time (MST)} = \frac{\sum \text{Survival time (days) of each mouse in a group}}{\text{Total number of mice}}$$

$$\text{Percent increase of life span (ILS) \%} = \left(\frac{\text{MST of treated group}}{\text{MST of control group}} - 1 \right) \times 100$$

Determination of haematological parameter

Haematological parameters were studied in both normal and tumour bearing mice. In case of tumour bearing mice, treatment was started after 24 hours of EAC cell transplantation and continued for 10 days. Blood was drawn from each group of mice on day 12 by tail puncture and determined Haemoglobin, Red blood cell and White blood cell content.

Total Red blood cell count

Exactly 10 μ l non coagulating blood was drawn with the tip of a micropipette and diluted to 1000 times with red cell counting fluid. RBC was counted with hemocytometer and the number of cells was counted with a microscope.

Total White blood cell count

Exactly 10 μ l non coagulating blood was drawn with the tip of a micropipette and diluted with 1 ml WBC counting fluid and mixed properly; the resultant mixture was checked in Neubauer haemocytometer and the number of cells was counted with a microscope. The dilution factor was 100. Total WBC cells per ml were calculated.

Estimation of haemoglobin

The amount of haemoglobin was measured by using Sahling's hemometer. Blood was drawn into the pipette up to the mark and transferred to the cuvette (tube) in haemometer containing a little amount of N/10 HCl. Distilled water was added and stirred until a good colour match was obtained. The final reading of the solution in the cuvette was noted. From the cuvette reading gram % (gm/dl) of haemoglobin was calculated.

RESULTS

Effects on cell growth inhibition

Effect of the extracts and bleomycin on the EAC cell growth on day six after tumour transplantation are shown in the figure 1.

Treatment with ethanolic extract of NP resulted in maximum cell inhibition at the doses 100mg/kg (i.p) and 50mg/kg (i.p) as evident from 76.74% and 40.35% reduction of tumour cells respectively whereas the same extract with formalin treated show marked reduction of cell growth inhibition as 52.69% and 29.35% respectively. The normal mango flesh show activity of some extent but the activity of formalin treated flesh is negligible. Bleomycin (0.3mg/kg) showed cell growth inhibition by 86.28% (data not shown in the figure).

Effects on survival time

The ethanolic extract of normal mango peel shows a significant increase on survival time of EAC cell bearing mice are shown in the figure 2. It has been observed that tumour induced mouse treated with

the ethanolic extract of normal mango peel at doses of 100 mg/kg (i.p) and 50mg/kg (i.p) resulted in increase of life span significantly which are 76.00 % and 44.79 % respectively, when compared to that of control mice. The formalin treated mango peel show moderate degree of increase life span 57.29% and 28.13% at doses of 100 mg/kg (i.p) and 50mg/kg (i.p) (figure 3) whereas the effect of other extract is not significant. The survival time increased when the dose of the extract is increased but treatment with formalin treated extract decreases the life span as compared to normal extract. Bleomycin increased the life span by 84.38% (data not shown in the figure) when compared to the control.

Effects on haematological parameter

Haematological parameters were found to be altered from normal values along with the growth of tumour. Haemoglobin and RBC count were found to be decreased and WBC count increased after the inoculation of EAC cells in Swiss albino mice. After treatment with the test extracts at doses 50 mg/kg (i.p) and 100 mg/kg (i.p), it was found that the parameters restored moderately only at high doses.

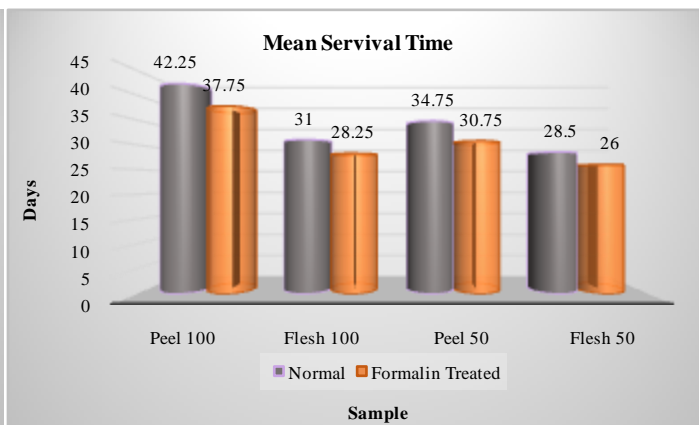
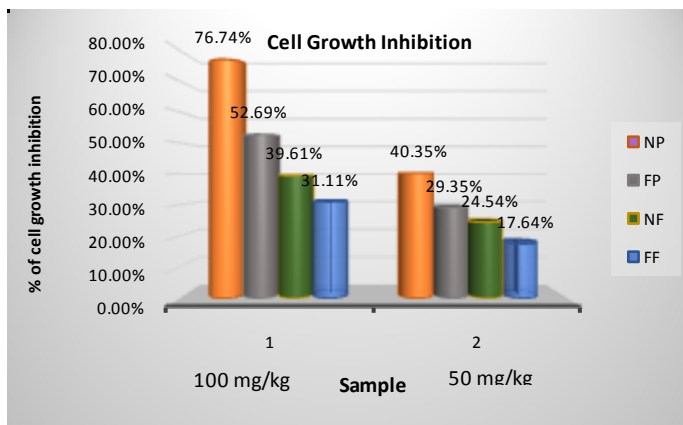


Figure 1: Percentage of cell growth inhibition on day 6 after tumour transplantation

Figure 2: Mean survival time of Swiss albino mice after treated with mango extract

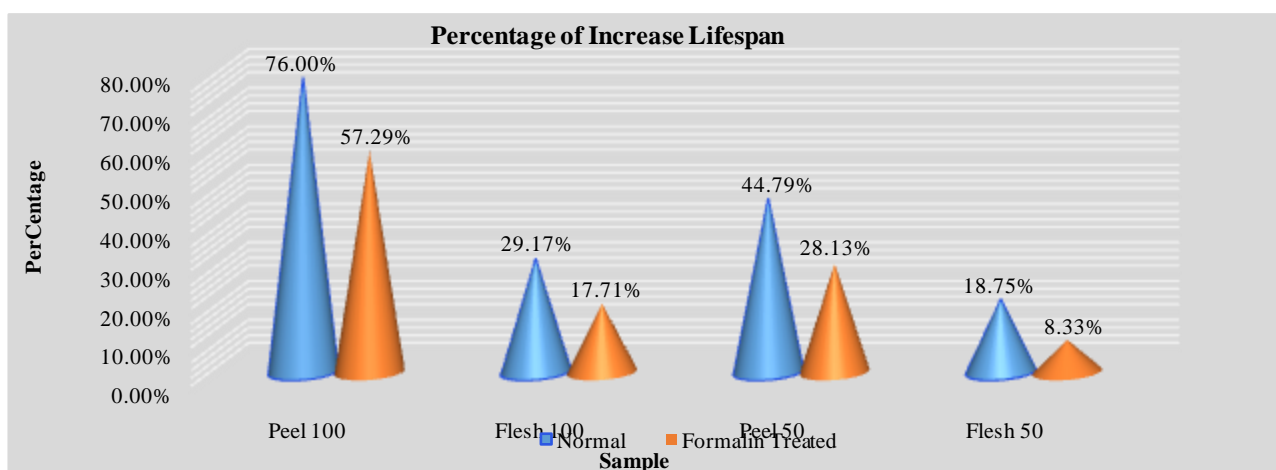


Figure 3: Percentage of increased life span of Swiss albino mice. Here the dose is expressed as mg/kg body weight

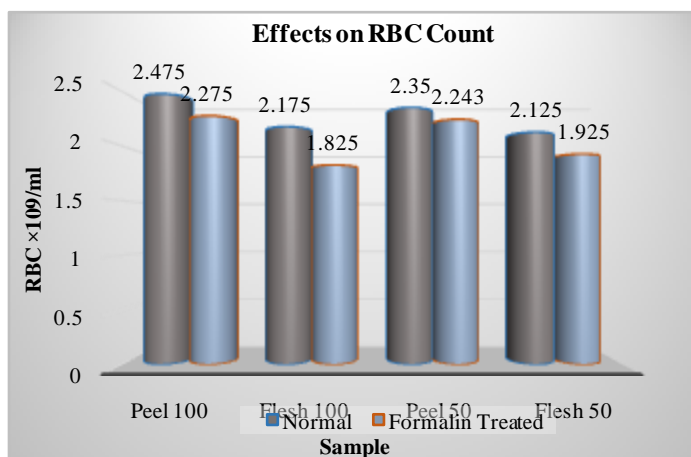


Figure 4: Effects of normal and formalin treated mango peel and flesh on RBC count

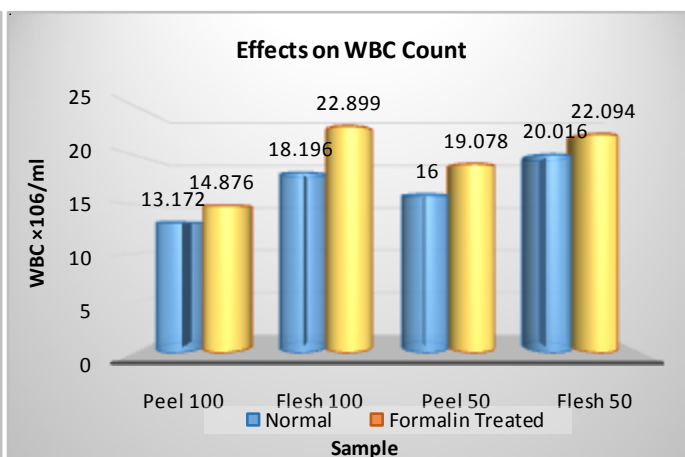


Figure 5: Effects of normal and formalin treated mango peel and flesh on WBC count

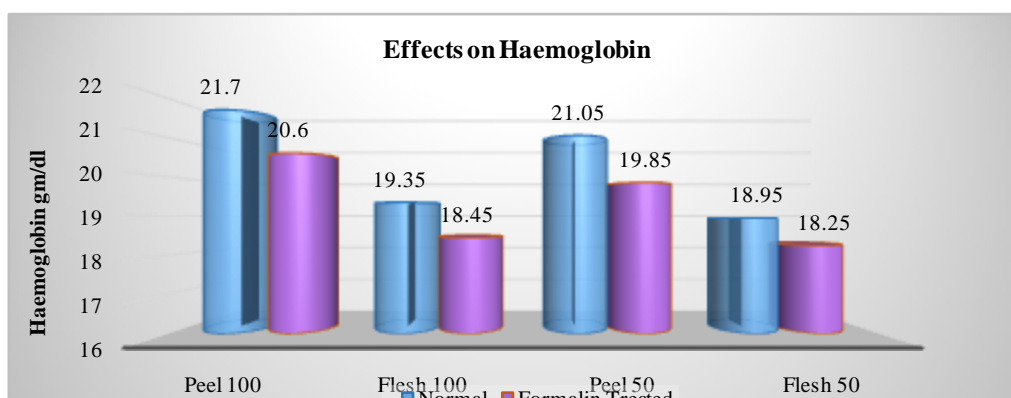


Figure 6: Effects of normal and formalin treated mango peel and flesh on haemoglobin content

DISCUSSION

It has been reported that uncontrolled proliferation of tumour cell decreases the RBC and Hb content; on the other hand, increases the WBC content [28-29]. To act as a chemotherapeutic agent, the compound must contain some of the property such as to inhibit the tumour growth, increase life span and also to correct the haematological parameters [30-31].

In the cell growth inhibition study, normal mango peel (NP) causes maximum inhibition of tumour cell growth among the extract and its inhibition activity increases with increase of dose. As compared to mango peel and flesh it usually finds that mango peel contains antioxidant activity greater than mango flesh [32-33], but when we compare it with formalin treated sample it shows that after treatment with formalin anticancer potentials decreases, which might be suggests that formalin penetrated to the peel of mango and changed the anticancer potential of it. Literature review suggests penetration of formalin in to the specimen is a physical process by which the solution diffuses in to the specimen to reach the innermost layers of cells [34] and when tissues are immersed in formalin, they are rapidly penetrated [35-37].

The survival time of tumour bearing mice is also in increased highest amount in normal mango peel (NP) which is satisfactory as compared to the standard drug bleomycin. The fleshes of both normal and formalin treated have failed to exert satisfactory increase of life span.

From the haematological study it is observed that, the red blood cell (RBC) and haemoglobin (Hb) is decreased after introduction of EAC cell where as white blood cell (WBC) shows the opposite trend. But treatment with the extracts the RBC is increased except formalin treated flesh (FF) (Figure 4). Normal mango peel (NP) at 100 mg/kg cause maximum increase. Mice treated with NP has RBC is about $(2.475 \pm 0.035) \times 10^9$ where in the normal mice its level is $(3.125 \pm 0.035) \times 10^9$. After treatment with EAC cell, WBC is increased from the normal value, but when treatment is started it decreases. But in case of formalin treated flesh (FF) it fails to show appreciable change. Normal mango peel (NP) at 100 mg/kg causes maximum reduction of WBC and the value is $(13.172 \pm 0.11) \times 10^6$ where the normal mice contain $(10.153 \pm 0.048) \times 10^6$ WBC (Figure 5). Normal peel (NP) at 50 mg/kg and formalin treated peel (FP) at 100 mg/kg also shows appreciable reduction of WBC. In case of haemoglobin, the introduction of EAC cell decreases Hb content and after treatment by mango extract in all cases it increases and the increase pattern is dose related.

CONCLUSION

This is the first study about comparative evaluation of anticancer property of mango after formalin treatment. There are several studies about penetration of formalin on animal tissue [35-37] but to our knowledge, there is no data about penetration of formalin in fruits.

From this study, it can be concluded that mango peel is a potent source of antineoplastic agent which is due to the presence of a phenolic compound, mangiferin [23-26]. From antineoplastic study, it is found that treatment with formalin markedly reduced antineoplastic activity which may be due to the reduction of antioxidant compounds [38-41].

Acknowledgement

We would like to give thanks to Ministry of education, Bangladesh for their financial support and also to the Pharmacy Department, Rajshahi University for technical support.

REFERENCES

- Hartwell J.L. Plants used against Cancer. *Lloydia* 1971; 32:204-255.
- Abdullaev F.I., Rivera L.R., Roitenburd B.V., Espinosa A.J. Pattern of childhood cancer mortality in Mexico. *Archives of Medical Research* 2000; 31(5):526-531.
- Jemal A., Bray F., Center M.M., Ferlay J., Ward E., Forman D. Global cancer statistics. *Cancer journal for clinicians* 2011; 61(2):69-90.
- Lozano R. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; (9859): 2095-2128.
- International Agency for Research on Cancer. World cancer burden 2012. http://publications.cancerresearchuk.org/downloads/Product/CS_REPOR_T_WORLD.pdf. (12 April 2016).
- Cancer statistics at a glance. <http://www.cancer.ca/en/cancer-information/cancer-101/cancer-statistics-at-a-glance/?region=on>. (12 April 2016).
- Canadian Cancer Statistics 2012. <http://www.phac-aspc.gc.ca/cd-mc/cancer/ccs-scc-2012-eng.php>. (12 April 2016).
- Siegel R.L., Miller K.D., Jemal A. *Cancer Statistics, 2015*. *A Cancer Journal for Clinicians* 2015; 65:5-29
- Cancer in Australia. <http://www.cancer.org.au/about-cancer/what-is-cancer/facts-and-figures.html>. (12 April 2016).
- Sasco A.J., Secretan M.B., Straif K. Tobacco smoking and cancer: a brief review of recent epidemiological evidence. *Lung Cancer* 2004; 45(2):3-9.
- Biesalski H.K., Mesquita B.B., Chesson A., Chytil F., Grimble R., Hemm R.J., Köhrle J., Lotan R., Norpoth K., Pastorino U., Thumham D. European Consensus Statement on Lung Cancer: risk factors and prevention. *Lung Cancer Panel. A Cancer Journal for Clinicians* 1998; 48(3):167-176.
- Schütze M., Boeing H., Pischon T., Rehm J., Kehoe T., Gmel G., Olsen A., Tjønneland A.M., Dahm C.C., Overvad K., Clavel-Chapelon F., Boutron-Ruault M.C., Trichopoulou A., Benetou V., Zylis D., Kaaks R., Rohrmann S., Palli D., Berrino F., Tumino R., Vineis P., Rodríguez L., Agudo A., Sánchez M.J., Dorransoro M., Chirlaque M.D., Barricarte A., Peeters P.H., van Gils C.H., Khaw K.T., Wareham N., Allen N.E., Key T.J., Boffetta P., Slimani N., Jenab M., Romaguera D., Wark P.A., Riboli E., Bergmann M.M. Alcohol attributable burden of incidence of cancer in eight European countries based on results from prospective cohort study. *Bio Med Central* 2011; 342:1584.
- Irigaray P., Newby J.A., Clapp R., Hardell L., Howard V., Montagnier L., Epstein S., Belpomme D. Lifestyle-related factors and environmental agents causing cancer: an overview. *Biomedicine and Pharmacotherapy* 2007; 61(10):640-658.

14. Kushi L.H., Byers T., Doyle C., Bandera E.V., McCullough M., McTieman A., Gansler T., Andrews K.S., Thun M.J. American Cancer Society Guidelines on Nutrition and Physical Activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. *A Cancer Journal for Clinicians* 2006; 56(5):254-281.
15. Park S., Bae J., Nam B.H., Yoo K.Y. Aetiology of cancer in Asia. *Asian Pacific Journal of Cancer Prevention* 2008; 9(3): 371-380.
16. Brenner H., Rothenbacher D., Amdt V. Epidemiology of stomach cancer. *Methods in Molecular Biology* 2009; 4:467-477.
17. Pagano J.S., Blaser M., Buendia M.A., Damania B., Khalili K., Raab-Traub N., Roizman B. Infectious agents and cancer: criteria for a causal relation. *Semin. Cancer Biology* 2004; 14(6):453-471.
18. Samaras V., Rafailidis P.I., Mourtoukou E.G., Peppas G., Falagas M.E. Chronic bacterial and parasitic infections and cancer: a review. *The Journal of Infection in Developing Countries* 2010; 4 (5):267-281.
19. Roukos D.H. Genome-wide association studies: how predictable is a person's cancer risk? *Expert Review of Anticancer Therapy* 2009; 9(4):389-392.
20. Maltoni C.F.M., Holland J.F. Holland-Frei Cancer Medicine. Chapter 16: Physical Carcinogens. In Bast RC, Kufe DW, Pollock RE 5th ed. Decker. Hamilton, Ontario 2000.
21. Formalin in fruits. *The Daily Star*, 12 June 2013. <http://archive.thedailystar.net/beta2/news/formalin-in-fruits/>. (26 February 2014).
22. Almost all litchis formalin-tainted. *The Daily Star*, 12 June, 2014. (12 June 2014).
23. Peng Z.G., Luo J., Xia L.H., Chen Y., Song S. CML cell line K562 cell apoptosis induced by mangiferin. *Zhongguo Shiyen Xue Ye Xue Za Zhi* 2004; 12 590-594.
24. Yoshimi N., Matsunaga K., Katayama M., Yamada Y., Kuno T., Qiao Z. The inhibitory effects of mangiferin: A naturally occurring glucosylxanthone, in bowel carcinogenesis of male F344 rats. *Cancer Letters* 2001; 163:163-170.
25. Noratto G.D., Bertoldi M.C., Krenek K., Talcott S.T., Stringheta P.C., Talcott S.U.M. Anticarcinogenic effects of Polyphenolics from mango (*Mangifera indica*) varieties. *Journal of Agricultural and Food Chemistry* 2010; 58(7):4104-4112.
26. Kim H., Kim H., Mosaddik A., Gyawali R., Ahn K.S., Cho S.K. Induction of apoptosis by ethanolic extract of mango peel and comparative analysis of the chemical constituents of mango peel and flesh. *Food chemistry* 2012; 133:416-422.
27. Sur P., Ganguli D.K. Tea plant extract (TRE) as an antineoplastic agent. *Planta Medica* 1994; 60:106-109.
28. Hurter B., Bush N.J. Cancer-related anemia: Clinical review and management update. *Clinical Journal of Oncology Nursing* 2007; 11:349-359.
29. Rosenbaum E.H. Anaemia causes and Treatment. *Cancer Supportive Care* 2008; 12:67-72.
30. Khanam J.A., Islam M.F., Jesmin M., Ali M.M. Antineoplastic activity of acetone semicarbazone (ASC) against Ehrlich ascites carcinoma (EAC) bearing mice. *Journal of the National Science Foundation of Sri Lanka* 2010; 38:225-231.
31. Perveen S., Islam M.F., Khanam J.A., Yeasmin T. Preventive effect of ethanol extract of *Alpinia calcarata* Rosc on Ehrlich's ascitic carcinoma cell induced malignant ascites in mice. *Asian Pacific Journal of Tropical Medicine* 2012; 6:121-125.
32. Berardini N., Fezer R., Conrad J., Beifuss U., Carle R., Schieber A. Screening of mango (*Mangifera indica* L.) Cultivars for their Contents of flavonol O- and Xanthone C-glycosides, anthocyanins, and Pectin. *Journal of Agricultural and Food Chemistry* 2005; 53(5):1563-1570.
33. Schieber A., Ullrich W., Carle R. Characterization of polyphenols in mango puree concentrate by HPLC with diode array and mass spectrometric detection. *Innovative Food Science and Emerging Technologies* 2000; 1:161-166.
34. Grizzle W.E., Fredenburgh J.L., Myers R.B. Fixation of tissues. In: Bancroft JD, Gamble M, editors. *Theory and Practice of Histological Techniques*. 6th ed. Philadelphia, USA: Elsevier Limited; 2008. pp. 56-63.
35. Fox C.H., Johnson F.B., Whiting J., Roller P.P. Formaldehyde Fixation. *Journal of Histochemistry and Cytochemistry* 1985; 33:845-853.
36. Kieman J.A. Formaldehyde, formalin, paraformaldehyde and glutaraldehyde: What they are and what they do. *Microscopy Today* 2000; 1:8-12.
37. Mason J.T., O'Leary T.J. Effects of formaldehyde fixation on protein secondary structure: A calorimetric and infrared spectroscopic investigation. *Journal of Histochemistry and Cytochemistry* 1991; 39:225-229.
38. Ding M., Lu Y., Bowman L.L., Huang C., Leonard S., Wang L., Vallyathan V., Castranova V., Shi X. Inhibition of AP-1 and neoplastic transformation by fresh apple peel extract. *The Journal of Biological Chemistry* 2004; 279:10670-10676.
39. Olsson M.E., Andersson C.S., Oredsson S., Berglund R.H., Gustavsson K.E. Antioxidant levels and inhibition of cancer cell proliferation in vitro by extracts from organically and conventionally cultivated strawberries. *Journal of Agricultural and Food Chemistry* 2006; 54:1248-1255.
40. Wang S.Y., Feng R, Lu Y, Bowman L, Ding M. Inhibitory effect on activator protein-1, nuclear factor-kappa B, and cell transformation by extracts of strawberries (*Fragaria x ananassa* Duch.). *Journal of Agricultural and Food Chemistry* 2005; 53:4187-4193.
41. Nacz M., Shahidi F. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis* 2006; 41:1523-1542.

HOW TO CITE THIS ARTICLE

Parvez GMM, Mosaddik A. Evaluation of anticancer property of mango peel and flesh after formalin treatment. *The Journal of Phytopharmacology* 2016;5(3):112-116.