Omoregie, Eriyamremu, Kapur, Cell Mol Biol 2020, 66:2

Cellular and Molecular Biology

Research Article Open Access

Therapeutic Effects of Aqueous and Ethanolic Extract of Phyllanthus amarus on 1, 2 Dimethylhydrazine Induced Colon Carcinogenesis in Balb/C Mice

Omoregie FO[1*], Eriyamremu GE[1] and Kapur S[2]

[1]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

[2]Department of Biological Sciences, Birla Institute of Technology and Sciences, Pilani, Hyd.Campus, India

***Corresponding author:** Dr. Department of Biochemistry, Faculty of Life Sciences, Universityof Benin, Benin City, Nigeria, Tel: 0049345-5574909; E-mail: skapur@hyderabad.bits-pilani.ac.in

Received: July 08, 2020; **Accepted:** July 31, 2020; **Published:** August 03, 2020

Abstract

Context: Phyllanthus amarus is traditionally used for treating various infections, inflammation and cancer. The underlying pathological mechanisms of colon cancer remain elusive. Evaluation of the effects of medical plant extracts in animal models could provide us with important ameliorative potential and therapeutic mechanisms.

Objective: In the present study the effects of aqueous and ethanolic extract of Phyllanthus amarus on colon cancer in Balb/C Mice induced by 1, 2 Dimethylhydrazine was investigated.

Materials and Methods: 30 female Balb/C Mice of weight 18-30g were acclimatized for a week and randomized into 6 groups (5/ group). Group A (-DMH), Group B(+DMH) , Group C(DMH + 250mg/kg body weight of ethanolic extract of P.amarus), Group D(DMH+ 350mg/kg body weight of ethanolic of P.amarus), Group E (DMH + 250mg/kg body weight of Aqueous extract of P.amarus), Group F(DMH+ 350mg/kg body weight of aqueous Extract of P.amarus). 20mg/kg body weight of DMH was administered orally for 21 days (twice a week). The plant extracts were administered daily for 3 weeks with the aid of a gavage immediately after colon cancer induction. Colon cancer was evaluated by the formation of Aberrant Cryptic Foci in the colon of DMH treated mice.

Results: Administration of the plant extracts (aqueous and ethanolic) ameliorated the carcinogenic effect of DMH in the colon of DMH treated mice in a dose dependent manner by significantly reducing the number of aberrant cryptic foci formed in extract-treated mice by 38% for 350mg/kg body of ethanolic extract and by 22% for 350mg/kg body of aqueous extract of P amarus.

Conclusion: The studied extracts had ameliorative potential on DMH induced colon cancer in Balb/C mice in a dose dependent manner providing evidence for the traditional use of this herb for treatment/prevention of cancer. Notably, 350mg/kg body of both extracts showed better reduction of AFC compared to 250mg/kg body of both ethanolic and aqueous extracts of P amarus.

Keywords: DMH (Dimethylhydrazine); *Phyllanthus amarus*; Aberrant Cryptic Foci (ACF); Carcinogen

Introduction

The term medicinal plant includes a variety of plants used in herbal therapy owing to their medicinal properties. Medicinal plants are considered to be a rich source of phytochemicals and bioactive components which can be used in drug development and synthesis; hence they are recommended for their therapeutic value [1]. The Genus Phyllanthus (Family: Phyllantheae) consists of approximately 1000 species, spread over the American, African, Australian and Asian Continents [2]. _Phyllanthus amarus is one of the most pharmacologically important species of the Phyllanthus family. It is a medicinally important plant also otherwise known as “stone breaker”, “carry me seed” etc. P amarus is an erect annual herb of not more than one and half feet tall. It has small leaves and yellow flowers. It is commonly found in forest areas, arid land, savannah areas, leached and exhausted soil in many countries including China, India, Nigeria, Cuba and Philippines amongst others [3,4]. Most of the herbs, belonging to the Phyllanthus family, afford various secondary metabolites conferring important medicinal properties. Bioactives such as alkaloids, flavonoids, lignin, phenols, tannins and terpenes have been isolated from these plants [5,6]. Due to its impressive preclinical therapeutic properties, extracts of species of the Phyllanthus have been evaluated to treat hypertension, jaundice, and diabetes. Other studies revealed preclinical pharmacological activity and therapeutic potential of phytochemicals isolated from P amarus [7]. The powdered leaves of P amarus (Bahupatra) were used in clinical studies evaluating its usefulness in patients suffering from chronic damage to the liver due to protracted hepatitis B virus infection. The powdered leaves were given in form of capsules to patients with chronic viral Hepatitis B in a dose of 200mg three times a day for 30 days and the treated patients tested negative for the viral antigen 15-20 days after the end of the treatment. Based on its very useful medicinal properties, P amarus is very frequently utilized in traditional medicine [8].

Cancer is a large group of disorders characterized by uncontrolled cellular proliferation. Cancer cells are also capable of metastasizing to other regions causing a number of devastating outcomes [9]. Nearly all body organs are vulnerable to cancer with liver, colon, and breast being the most common ones. Colon cancer is a type of cancer that begins in the large intestine (colon). The colon is the final part of the digestive tract. Colon cancer has an estimated incidence of over 1 million new cases annually worldwide [10]. Almost one of three patients with colon cancer dies from the disease. Colon cancer also more often affects people of well-developed countries in comparison to less developed countries [11]. Colorectal cancer is one of the leading causes of tumor-related death and despite its high prevalence, the underlying pathological mechanism remain elusive [9]. Colorectal cancer is a multistep process affected by environmental and genetic factors which lead to normal colonic epithelium to dysplasia followed by a benign precursor stage, the pre-malignant polyp and can progress to invasive disease. Besides a genetic pre-disposition, diet also determines the risk for colon cancer and predominantly diets rich in fruit and vegetable diminish the risk of the disease [10].

In the present investigation, 1,2 Dimethylhydrazine -induced model was utilized, as it is similar to histopathological and molecular characteristics of the human colon cancer model [11]. 1,2 Dimethylhydrazine is metabolized in the liver to form Azoxymethane and methylazoxymethanol later transported to the colon via bile or blood to generate its ultimate carcinogenic metabolite, diazonium ion which elicits oxidative stress by methylating biomolecules of the colonic epithelial cells thus leading to promutagenic events, inflammation and tumors in the colon [12].

Recently, interest in the search of naturally occurring antioxidants from plants has been rekindled. The current research work investigated the ameliorative ability of aqueous and ethanolic extract of P amarus leaves on 1,2 Dimethylhydrazine induced colon carcinogenesis in mice model.

Aim of Study

This study aims to evaluate the therapeutic effects of aqueous and ethanolic extract of P amarus leaves on 1,2 Dimethylhydrazine induced colon cancer in Balb/C mice.

Materials and Methods

Plant collection

The leaves of P amarus were collected from the botanical garden of University of Benin, Nigeria and were identified by an expert in the Department of Botany, University of Benin, Benin City.

Plant Sample Preparation

The leaves of this plant were air-dried in the laboratory at the Department of Biochemistry, University of Benin, Benin City. The leaves were later pulverized to powdery form in Pharmacognosis laboratory at the Faculty of Pharmacy, University of Benin. Ca. 250g of the powdered leaves of P amarus was soaked in 1.5 liters of absolute ethanol for 24 hour with periodic stirring of the mixture. After 24 hour, the mixture was filtered with fine cheese cloth, the residue was discarded and the filtrate was used to soak another 250g of powdered leaves of same plant above, allowed to stand for another 24 hour with continuous stirring, thereafter, the mixture was again filtered. The residue was discarded and the filtrate was filtered with Whatman filter paper (No: 1) and was concentrated with the aid of a vacuum concentrator at 30°C. The concentrates were then weighed and used as experiment sample. The same procedure was carried out with distilled water for the aqueous extract. The above isolation of crude extract was done at the Department of Biological Sciences, Birla Institution of Technology and Science, BITS-Pilani, Hyderabad, India.

Phytochemical screening

Phytochemical screening to detect the presence of bioactive agents was performed by the procedures described [13,14]. After the addition of specific reagents to the solution, the tests were detected by visual observation of color change or by precipitate formation.

Chemical: DMH was purchased from TCI Chemical, Chennai.

Animal Study: 30 female Balb/C Mice of weight 18-30g were purchased from VAB Biosciences, Bapuji Nagar, Musheerabad, Hyderabad-500020.They were maintained according to the Institutional Animal Guidelines (1912/PO/Re/S/16/CPCSEA) and acclimatized to diet and environment for 1 week after arrival. They were housed in a density of 5 animals per rack mounted plastic with detachable steel aerated covered cages and were given clean drinking water ad libitum. The temperature (20-22°C) and lighting (12 hour light/dark cycle) were constantly controlled. DMH was dissolved in Millipore water and was administered (20 mg/kg body) orally. The volumes of gavage were from 0.18-0.3 ml. _Oral administration of DMH lasted for 21 days (twice a week). Upon completion of the doses of carcinogen, the DMH treated mice were randomized in 5 groups, groups B – F. Group B served as positive control, Group C received 250mg/kg body ethanolic extract of P.amarus, Group D received 350mg/kg body weight of ethanolic extract of P.amarus, Group E received 250mg/kg body weight of aqueous extract of P.amarus and Group F received 350mg/kg body weight of aqueous extract of P.amarus. The plant extracts were administered daily as an oral gavage for 21 days.

Analysis of Aberrant Cryptic Foci: Aberrant cryptic foci (ACF) were analyzed at the end of the experiment using procedure described [15]. The animals were sacrificed by cervical dislocation and their colons were removed and flushed with Kreb's ringer salt solution. _The colons were cut open along the longitudinal axis and fixed flat between filter paper in 10% buffered-formalin solution for 24 hours and were stained with Methylene blue (0.05% in Kreb's ringer salt solution) for 30 minutes in order to visualize crypts' outlines. The colons were mounted on microscopic slides with the mucosal surface up and aberrant crypts were scored under a confocal microscope at a magnification of 20X. The number and location of the aberrant crypts were recorded. Aberrant crypts were distinguished from surrounding normal-appearing crypts based on 3 characteristics: increased size, significantly increased distance from the luminal to basal surfaces of cells, and the easily discernible pericryptal zone.

Statistical Analysis

Counts of ACFs were expressed as mean ± standard error of mean (SEM). All statistics were computed using Microsoft Excel & Graph pad prism 7. Values of $p<0.05$ were considered significant.

Results

Phytochemical screening has revealed that the ethanolic extract contained tannins, saponins, flavonoids, quinines, phenols, terpenoids, and phyto steroids except glycosides (Table 1). The aqueous extracts consisted for tannins, saponins, flavonoids, glycosides and phenols but were devoid of terpenoids and phyto steroids. The difference in the phytochemical composition of the ethanolic and aqueous extract could be due to differential solubility of the constituent phytochemicals.

Phytochemicals	Ethanol	Aqueous
Tannin	+	+
Saponins	+	+
Flavonoids	+	+
Glycosides	-	+
Quinones	+	+
Phenols	+	+
Terpenoids	+	-
Steroids (phytosteroids)	+	-
Key: + for present and - for absent		

Table 1: Phytochemical Screening.

Highest weigh gain of 9.21g was observed after the experimental period of 21 days in the negative control (Group A) that did not receive any type of induction treatment nor received any type of extracts as feed (Table 2, Figure 1). Group B mice functioned as positive control which were treated with DMH but were not fed with any type of plant extracts. The group B mice showed least mean increase by 1.87g in their body weight. This is because there was no protection by the plant extracts. The weight gains were similar among Group C DMH treated mice administered with 250 mg/kg BW of ethanolic extract and Group E DMH treated mice administered with 250 mg/kg BW of aqueous extract. However, when the daily extract dose was increased to 350 mg/kg BW to DMH treated mice, the aqueous extracts resulted in significantly higher weight gain by 3.94 g in Group F when compared to 2.24g in Group D (Table 2).

	Group A	Group B	Group C, DMH	Group D, DMH	Group E, DMH	Group F, DMH
Weight	(-ve cont.)	(+ve cont.)	250mg/kgbwt.Et P.a	350mg/kgbwt Et P.a	250mg/kgbwtAq P.a	350mgkgbwtAq P.a
Initial wt.	31.30 ± 0.82	29.80 ± 1.01	19.03 ± 0.75	19.47 ± 0.81	18.82 ± 0.82	19.03 ± 0.32
Final wt.	40.47 ± 2.20	31.67 ± 2.85	21.93 ± 1.09	21.71 ± 1.86	21.66 ± 3.67	22.97 ± 1.21
Wt gain	9.21	1.87	2.92	2.24	2.84	3.94

Table 2: Data showing the Weight of animals.

Highest number of aberrant cryptic foci was noticed in Group B mice that were subjected to DMH treatment but however did not receive any type of protection from any type of plant extract (Table 3). Least number of ACF (13.33) were observed in Group D colon cancer induced mice treated with a daily dose of 350 mg/kg BW of ethanolic extract of P. amarus for a period of 21 days indicating higher protection and potential in percent reduction of ACF (Table 3, Figure 2). The ACF in mice treated with 250 mg/kg BW of either aqueous or ethanolic extract remained almost similar. However, at a feed concentration of 350 mg/kg BW, the ethanolic extract of P. amarus was more effective with concomitantly reduced level of 13.33 ACF than the aqueous extract with 16.66 ACP. A representative microscopic image of the normal crypts and aberrant cryptic foci along with their characteristic features are shown in Figure 3.

Groups	Treatment	Number of ACF	Number of Crypts/focus
Group A	-DMH	—	—
Group B	+DMH	21.67 ± 4.71	1
Group C	DMH+250mg/kgbwt (Etoh of P.amarus)	19.50 ± 2.04	1
Group D	DMH+350mg/kgbwt (Etoh of P.amarus)	13.33 ± 2.33	1
Group E	DMH+250mg/kgbwt (Aq of P.amarus)	19.00 ± 1.01	1
Group F	DMH+350mg/kgbwt (Aq of P.amarus)	16.66 ± 2.03	1

Table 3: Data showing number of aberrant cryptic foci in the colons of Experimental animals.

With respect to the location and distribution of ACF, it was found that ACF were most densely present (10.33) in distal end followed by mid colon (4.67) and proximal end (6.678) of the mice colon in Group B mice that were treated with DMH but did not receive any extract in feed (Table 4). At a feed level of 250 mg/kg BW, the distal end of the colon showed significantly higher number of ACF in mice fed with ethanolic extract than that in mice fed with aqueous extract. At 350 mg/kg BW ethanolic extract concentration, the mid and distal end showed lesser number of ACF while their density was higher at proximal end of the colon. This is in contrast to the effect of aqueous extract at 350 mg/kg BW concentration.

Groups	Proximal	Mid	Distal
GroupA (-DMH)	—	—	—
GroupB (+DMH)	6.67 ± 1.33	4.67 ± 1.77	10.33 ± 3.69

GroupC (DMH, 250mg/kgbwt Et. P.amarus)	1.00 ± 0.82	6.00 ± 1.63	12.50 ± 2.86
GroupD (DMH, 350mg/kgbwtEt. P.amarus)	7.00 ± 2.08	2.00 ± 0.99	4.33 ± 2.03
GroupE (DMH, 250mg/kgbwtAq. P.amarus)	1.50 ± 0.50	9.00 ± 1.01	8.50 ± 0.70
GroupF (DMH, 350mg/kgbwtAq. P.amarus)	3.33 ± 0.33	7.33 ± 1.20	6.00 ± 0.58

Table 4: Data Showing Number of aberrant cryptic foci /Location in Mice Colon.

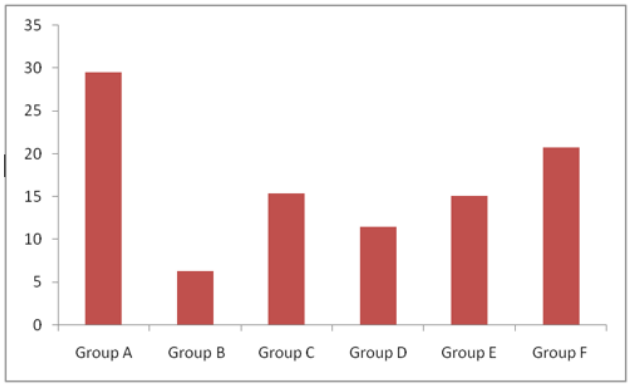

Figure 1: Showing percentage weight gain in experimental animals.

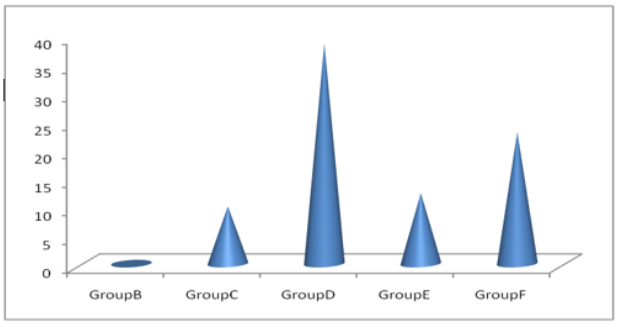

Figure 2: Showing percentage reduction of ACF in extract treated colon.

Discussion

Plant bioactive components have the potential to be major chemo-protective ingredients to control cancer. Substantial evidence indicates that plant bioactive components may play an essential role in colon cancer prevention and management [16]. In this study, the therapeutic effects of aqueous and ethanolic extract of P amarus was investigated for their anti-colon cancer ability in Balb/C mice. It holds an array of secondary metabolites with medically important properties. Bioactives such as alkaloids, flavonoids, lignin, phenols, tannins and terpenes have been isolated from this plant [5,6].

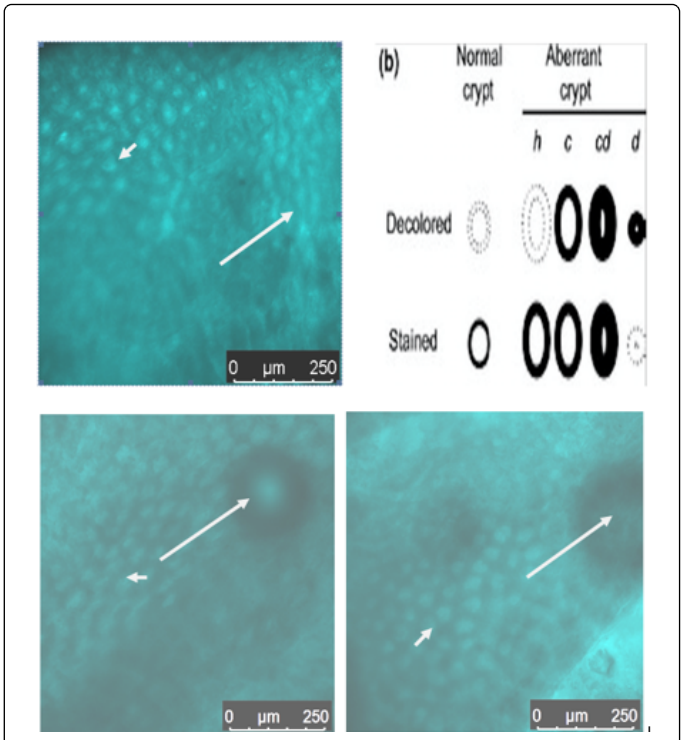

Figure 3: Showing microscopic view of Mice colon with ACF at a magnification of 20X under confocal microscope. (b) Schematic view of the crypt subtypes. A ring-shaped image represents the morphology of epithelial lining [31].

The results from this study show that P.amarus has therapeutic properties against colon cancer induced by DMH. Its medical properties could be as a result of the presence of useful phytochemicals and antioxidant such as flavonoids, polyphenols, alkaloids and tannin. They are major contributors to anticancer and anti-hepatotoxic capability of most medicinal plants [17]. Numerous epidemiological studies have validated the inverse relation between the consumption of flavonoids and the risk of cancer. _Flavonoids possess cancer blocking and suppressing effects, they are involved in the regulation of enzymes of phase - II responsible for xenobiotic biotransformation and colon microflora [18]. Colon cancer is a major cause of morbidity and mortality throughout the world [19]. It accounts for over 9% of all cancer incidences [20,21]. It is the third most common cancer worldwide and the fourth most common cause of death [21]. It affects men and women almost equally, with over 1 million new cases recorded in 2002, the most recent year for which international estimates are available [19,20,22-24]. Countries with the highest incidence rates include Australia, New Zealand, Canada, the United States, and parts of Europe. The countries with the lowest risk include China, India, and parts of Africa and South America [20].

Some effect of P. amarus was also seen on the weight of the experimental animals. Groups treated with the plant extracts had increase weight relatively close to negative control (-DMH) group, but significantly higher than the weight of positive control (+DMH) group. This could be due to the phyto-nutrients (vitamins and minerals) present in the plant extract [25-27]. The therapeutic effect of P.amarus in colon cancer could also be linked to the secondary metabolite, phenolic acid. Phenolic acid is implicated for its ant-proliferative and

pro-apoptotic effects in colon cancer cell line in a concentration dependent manner [28]. Lignin a bioactive in P.amarus also contributes to the prevention of colon cancer. The mechanism of action is the ability of colon bacteria to convert it into biologically active lignans such as enterodiol and enterolactone. These lignans are structurally similar to estradiol and therefore, they exert anticancer effects on hormone-related cancer [29,30].

Aberrant cryptic foci are useful intermediate biomarkers in detecting modifying influences of natural and synthetic compounds on chemically induced colon carcinogenesis, which represents the preneoplastic lesions [25]. Cell proliferation, plays an important role in multistage carcinogenesis with multiple genetic changes [26]. Modulation of cell-proliferation activity in target organs is one of the important actions of cancer chemoprevention [27].

In the current study, DMH induced aberrant cryptic foci in all the groups treated with the cocktail (DMH) after 3 weeks of administration. This effect was significant and dose dependent as observed in the groups treated with different doses as summarized in Table 3 and 4 [31]. Ethanolic extract at concentration 350 mg/kg body weight had a more significant reduction on ACF formation in the colon than its counterpart (aqueous extract) at the same concentration. It is noteworthy that treatment with both extracts at 350mg/kg body weight significantly reduced ACF formation. Though there was mild reduction in colon treated with 250mg/kg body weight, but the difference was insignificant.

Conclusion

This preliminary study was to investigate the therapeutic effects of aqueous and ethanolic extracts of Phyllanthus amarus on 1, 2 Dimethylhydrazine induced colon carcinogenesis in Balb/C mice. The overall results suggest that the plants extract had preventive and protective effects on DMH induced colon carcinogenesis in Balb/C mice. Notably, 350mg/kg body weight of both extracts showed higher bioactivity than 250mg/kg body weight and this bioactivity is higher in ethanolic extract in comparison to the aqueous extract at the same concentration.

Acknowledgment

This work was fully funded by the Department of Science and Technology (Government of India), sanction of Federation of Indian Chambers of Commerce and Industry (FICCI). Research Training Fellowship-Developing Country Scientist (DCS2018/000063). We gratefully acknowledge the support provided by genomic lab at Birla Institute of Technology and Science, Especially Mr. Pavan Muyawdiya in conducting the animal studies and biochemical analysis.

Conflicts of Interest

The Authors declare no conflict of interest.

References

1. Rasool, H. Medicinal Plant (Importance and uses). Pharmaceut Anal Acta., 2012; 3: 2153-2435.
2. Tasheen, M., Mishra, G. Ethnobotany and Diuretic Activity of some Selected Indian Medicinal plants.The Pharma Innovation., 2013; 2: 112.
3. Bharatiya, V.B. Selected medicinal plants of India Bombany. Tafa press., 1992; 2: 235-237
4. Burkill, H.M. The useful plant of West Tropical Africa. Royal botanic., 1994; 2: 234-237.
5. Bahar, L., Sarker, S.D., Delazar, A. Phytochemistry of the genus Phyllanthus, in Phyllanthus Species Scientific Evaluation and Medicinal Applications. Taylor and Francis Group CRC Press, London, United Kingdom., 2011 2: 119-138..
6. Calixto, J.B., Santos, A.R.S., Filho, V.C. A review of the plants of the Genus Phyllanthus their chemistry, pharmacology and therapeutic potential.Medical Research Reviews., 1998; 18: 225-238.
7. Araujo R.E., Luiz, A.S., Cinthia, R.C., Ranniere, G.F., Hugo, C.G., Pedro, R.P., et al. Growth Inhibitory effects of Phyllanthus niruri extracts in combination with cisplatin on cancer cell lines. World Journal of Gastroenterology., 2012; 18: 4162-6168.
8. Joseph, B., Raj, S.J. Pharmacognostic Properties of Phyllanthus amarus. International Journal of Pharmacology., 2011; 7: 40-45.
9. Son, DJ., Lee, JW., Lee, YH., Song, HS., Lee, CK., Hong JT. Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. Pharmacol Ther., 2007; 115: 246-270.
10. Cherbuliez, T. Apitherapy-the use of honeybee products. In: Grassberger M (ed.), Biotherapy-history, principles and practices. Springer, London, United Kingdom., 2013; 1: 113-146.
11. American Cancer Society. Global cancer facts & figures. 3rd edition, American Cancer Society, Atlanta, GA, 2015.
12. Swiderska, M., Choromanska, B., Dabrowska, E., Konarzewska, DE., Choromanska, K., Szczurko. The diagnostics of colorectal cancer. Contemp Oncol., 2014; 18: 1-6.
13. Sofowora, E.A. Medicinal plant and traditional medicine in Africa. John Wiley and Sons., New York., United States., 1982; 1: 1-10.
14. Trease, G.E. and Evans, W.C. Pharmacology 2 ed. Bailliere Tindall ltd, 1989., 1: 60-75, 1989.
15. Barbara, T., Ranjana, P.B., Robert, B. Foci of Aberrant Crypts in colon of Mice and Rats Exposed to Carcinogens Associated Food. American for Cancer research., 1989; 49: 1236-1240
16. Gwyn K, Sinicrope FA. Chemoprevention of colorectal cancer. Am J Gastroenterol.2002; 97:13-31.
17. Nwanna, E.E., Oboh, G. Antioxidants and Hepatoprotective properties of polyphenols extract from Telfaracia occidentalis Leaves. Pak J Biol Sci., 2007; 10: 2682-2687.
18. Anup,K., Sonia, G., Swati , K. Role for flavonoids at the cellular level. Cancer Phytotherapeutics., 2008; 22: 567- 577.
19. World Health Organization Cancer Incidence in Five Continents. Lyon: The World Health Organization and The International Agency for Research on Cancer; 2002.
20. Boyle, P., Langman JS. ABC of colorectal cancer: Epidemiology. BMJ., 2000; 321: 805-808.
21. World Cancer Research Fund and American Institute for Cancer Research Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective. Washington, DC: American Institute for Cancer Research; 2007.
22. Boyle, P., Ferlay J. Mortality and survival in breast and colorectal cancer. Nat Clin Pract Oncol., 2005; 2: 424-425.
23. Ferlay, J., Bray, F., Pisani, P., Parkin, DM. GLOBOCAN 2002: Cancer incidence, mortality and prevalence worldwide. Lyon: International Agency for Research on Cancer; 2004.
24. Parkin D., Bray F., Ferlay J. CA Cancer J Clin., 2006; 55: 74-108.
25. Takefumi, K., Keiji, H., Hideki, I., Nariaki, M., Shin-ichiro, S., Hideaki, I. Aged Garlic Extract has chemoprotective effects on 1,2 Dimethyldrazine-induced colon tumors in Rats. The Journal of Nutrition., 2006; 136: 847-851.
26. Cohen, SM. Cell proliferation and carcinogenesis. Drug Metab Rev.,1998; 30: 339-357.
27. Mori, H., Sugie, S., Yoshimi, N., Hara, A., Tanaka T. Control of cell proliferation in cancer prevention. Mutat Res., 1999; 428: 291-298.

28. Cen L, B., Hutzen, S., Ball. New structural analogues of curcumin exhibit potent growth suppressive activity in human colorectal carcinoma cells. BMC Cancer., 2009., 9: 1-9.

29. Xiong X.Y., Hu X.J., Li Y., Liu, C.M. Inhibitory effects of enterolactone on growth and metastasis in human breast cancer. Nutr Cancer 2015; 67: 1324-1332.

30. Yue, Z., Jie, Z., Ya, L., Dong-ping, X., Sha, L., Yu-Ming, C., et al. Natural Polyphenols and Prevention of Cancer. Canc., 2016; 8:515.

31. Masako, O., Yoshitaka, H., Masashi, I. Newly Defined Aberrant Crypt Foci as a Marker for Dysplasia in rat colon. Cancer Science; 2014., 105: 943-950.