World Applied Sciences Journal 32 (3): 444-450, 2014
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2014.32.03.85109

Evaluation of Anticancer Activity of *Annona muricata* in 1, 2-Dimethyl Hydrazine Induced Colon Cancer

[1]*Venkateshwarlu Eggadi,* [1]*Swathi Gundamedi,* [1]*Sharvana Bhava Bandaru Sheshagiri,*
[1]*Shiva Kumar Revoori,* [3]*Venkateshwar Rao Jupally and* [1]*Umasankar Kulandaivelu*

[1]Vaagdevi college of Pharmacy, Hanamkonda, Warangal, Telangana State, India-506002
[2]Singhania University Pacheri, Jhunjhun, Rajastan
[3]Department of Chemistry, Talla Padmavathi College of Pharmacy,
Ursu, Warangal, Telangana State, India-506009

Abstract: *Background:* Cancer is a major public health burden in both developed and developing countries. Anticancer activity is the effect of natural and synthetic or biological and chemical agents to reverse, suppress or prevent carcinogenic progression. Several synthetic agents are used to cure the disease but they have their toxicity and hence the research is going on to investigate the plant derived chemotherapeutic agents. Cancers are the second cause of death worldwide in the 21st century. Colon cancer is third most common cancer. The present study was conducted to evaluate *in vivo* anticolon cancer activity of *Annona muricata.* Materials and Methods: The anticolon cancer activity of *Annona muricata* against 1, 2-dimethyl hydrazine (DMH) induced colon cancer in Wistar albino rats. Results: The plant extract EEAM (Ethanolic extract of *Annona muricata*) at dose of 300 mg/kg significantly (P<0.001) decreasing the aberrant crypt foci (ACF), weight of individual organs, hematological parameters and significantly (P<0.001) increased weight gain and apoptosis index (P<0.05) when compared with disease control (DMH). The biochemical parameter total cholesterol level were also decreased significantly (P<0.01) when compared with disease control (DMH). We concluded that based on the above results Ethanolic extract of *Annona muricata* it has shows potent anti cancer activity.

Key words: EEAM-Ethanolic Extract of *Annona muricata* · ACF-Aberrant Crypt Foci · DMH-1, 2-Dimethyl Hydrazine

INTRODUCTION

Cancers are the second cause of death and have the highest burden of diseases in 21st century [1]. The annual cancer deaths will increase from 7.6 million in 2008 to 13 million in 2030, according to the WHO world health statistics report 2012. For the survival of any organism, there should be a delicate balance between the cell growth and death. This balance can get disturbed in a number of ways, which may lead to either abnormal growth of tissue [2] or may develops into a lethal tumor or cancer [3]. Cancer is a major public health burden in both developed and developing countries. Anticancer activity is the effect of natural and synthetic or biological and chemical agents to reverse, suppress or prevent carcinogenic progression. Several synthetic agents are used to cure the disease but they have their toxicity and hence the research is going on to investigate the plant derived chemotherapeutic agents.

Colon cancer is one of the most common malignancies in many regions of the world is thought to arise from the accumulation of mutations in a single epithelial cell of the colon and rectum. Colon cancer is the second leading cause of cancer related deaths in USA [4]. Recently colon cancer incidence has been increasing in India year by year [5].

Annona muricata Linn (*Annonaceae*) is commonly known as 'Soursop' or 'Graviola. It is grown in parts of Southeast Asia. Graviola produces natural compounds in its leaf, stem, bark and seeds [6]. The fruits, seeds and leaves are used to treat stomach ailments, fever, parasitic infections, hypertension and rheumatism [7]. Plant has

Corresponding Author: Venkateshwarlu Eggadi, Department of Pharmacology, Vaagdevi College of Pharmacy, Warangal, Telangana State, India-506001. Mob: +919848835092.

been used medicinally in many tropical African countries array of human ailments, especially for parasitic infections and cancer [8].

MATERIALS AND METHODS

Reagents and Chemicals: 1, 2-Dimethyl hydrazine (DMH) was produced from Sigma-Aldrich, Bangalore. Methylene blue from Sisco Research Laboratories private limited, Mumbai. Haematoxylin from Merck specialities private limited, Mumbai. All the solvents and chemicals were produced from SS Pharma, Hanamkonda and they were of analytical grade quality.

Plant Material: The leaves of *Annona muricata* were collected in June 2013 from the Hasanparthy region, Warangal District, The plant was identified and authenticated by Prof. Raju, department of Botany, Kakatiya University, Telangana state.

Preparation of Extract: The collected *Annona muricata* leaves were dried and powdered using mixer grinder. The dried plant material was macerated using maceration with ethanol for 3 x 24 hours. The powder was soaking 100 gm of dried powder in 200 ml ethanol for three days at room temperature with occasional shaking. Then it was filtered with filter paper and filter was evaporated to dryness in an evaporating dish at room temperature to obtain semi solid substance. The dried extract was weighed and stored in an air tight container for anti-cancer activity testing in rat models.

Animals: In this study Male Wistar albino rats (150-180 gm) were selected and procured from Mahaveer enterprises, Hyderabad, India. All animals were maintained under environmentally controlled conditions of 25±2° C, relative humidity of 45 to 55 % and 12 hr light-12 hr dark cycle. The animals were acclimatized to laboratory conditions at least one week before starting the experiment and they had free access to food and water *ad labitum*. The study protocol was approved by Institutional Animal Ethics Committee (IAEC NO: 1047/ac/07/CPCSEA).

Acute Toxicity Studies: Acute oral toxicity test was carried out according to the OECD guideline No. 423. Wistar Albino Rats were kept for overnight fasting prior to drug administration. A total of three animals were used, which received a single oral dose in 2000 mg/kg, body weight of CG chloroform extract. The animals were observed for a period of 24 hrs for the changes in behaviour, hypersensitivity reactions etc. Mortality, if any, was determined over a period of 2 weeks [9].

Experimental Design: Twenty four Wistar Albino Rats were used in this study.

The Rats were randomised and divided into four groups of six animals each.

Group I: Vehicle control: received normal saline
Group II: Disease control (DMH: 30 mg/Kg, *s.c*)
Group III: Disease + 5-Florouracil (5-FU: 20 mg/kg, *i.p*)
Group IV: Disease + EEAM (300 mg/kg, *p.o*)

***In vivo* Methods:**

Body Weight Changes: The body weight changes of the control, DMH, 5-FU and EEAM treated rats were measured throughout the study. The rats were weighed at the beginning of the experiment and then subsequently once a week and final before sacrifice [10].

Determination of Aberrant Crypt Foci (ACF): At the end of the study, rat colons were removed and flushed with Potassium Phosphate buffered saline (0.1M, pH 7.2) and colons were split open longitudinally and placed on strips of filter paper with their luminal surfaces open and exposed. Another strip of filter paper was placed on top of the luminal surface. The colons were then secured and fixed in a tray containing 10% buffered formalin for overnight. Each of the fixed colons was cut into proximal and distal portions of equal lengths and portion was further cut into 2cm long segments. Each segment was placed in a Petri dish and stained with 0.2% methylene blue solution for 2min.The segment were examined using a light microscope at low magnification to score the total number of ACF as well as the number of crypts per focus. ACF were distinguished from normal crypts by their thicker, darker-stained, raised walls with elongated slit-like lumens and significantly increased distance from the lamina to basal surface off cells [11].

Apoptosis Measurement in Colonic Mucosa: Apoptosis evaluation was carried out in paraffin-embedded section of normal colonic mucosa and tumours stained with haematoxylin. At least 20 full longitudinal crypt sections of normal mucosa of rat were scored at the microscope, determainining the presence of cells in each crypt with the following characteristics of apoptosis: cell shrinkage, loss of normal contact with the adjacent cell, chromatin condensation or formation of round or oval nuclear

fragments. When clusters of more than one apoptotic body were seen within the diameter of one cell, these bodies were considered as fragments of one apoptotic cell. Tumour apoptosis was determined by scoring at least 1000 cells/rat for the presence of apoptotic cells that were coded as described. In tumours and colon mucosa, apoptosis was scored by a single observer on coded samples and quantified as apoptotic index [12].

AI=number of apoptotic cells/cells scored×100.

Haematological Evaluation: The initiation of the study and immediately before necropsy, blood samples were collected for haematological analysis in EDTA tubes with 1.5%EDTA and differentially quantified through a coulter T890 for the following: leukocyte, erythrocyte, platelet counts and haemoglobin determination [13].

Individual Organs Weights: At the end of the study animals were sacrified and remove the all visceral organs like liver, kidney, colon, spleen, heart, pancreas, stomach, lungs were removed, weighed and relative weight of organs were calculated [14].

Biochemical Parameters: On the day of necropsy, approximately 5ml of blood was collected from the rats under mild anaesthesia and immediately centrifuged by cooling centrifuge at 3000 rpm for 15 min. Serum was collected and preserved frozen at -30°C and total cholesterol concentrations in the serum were determined enzymatically using commercially available reagent kits [15].

Histopathology of Colon: The colon were excised, flushed with saline, cut open longitudinally along the main axis and then again washed with saline. These colonic sections fixed in 10% buffered formalin for at least 24 hrs and after fixation, the specimens were dehydrated in ascending grades of ethanol, cleared in benzene and embedded in paraffin wax. Blocks were made and 5ìm thick sections were cut from the distal colon. The paraffin embedded colonic tissue sections were deparaffinised using xylene and ethanol. These sections stained with haematoxylin and eosin and were observed under light microscope at 10X and 40X magnifications to investigate the histoarchitecture of colonic mucosa [16].

Statistical Analysis: Results were expressed as Mean ± S.E.M, statistical significance was calculated by applying one way ANOVA. $P<0.05$ was considered as significant (Dunnett's Multiple Comparison Test).

RESULTS

Acute Toxicity Study: In the acute oral toxicity study, at a dose of 2000 mg/kg body weight of EEAM, mortality was not observed. From the results, test drug dose of 300 mg/kg body weight were chosen for the efficacy studies.

Weight Changes: During the experimental period (30days), the carcinogen-exposed rats exhibited a significantly low gain in body weight and growth rate throughout the experimental period as compared to groups I, III and IV when compared with DMH group (IV). The EEAM (300 mg/kg) and 5-FU (20 mg/kg) significantly ($P<0.001$) increased in weight gain when compared with DMH group respectively (Table 1).

Determination of Aberrant Crypt Foci (ACF): ACF formation was observed in all DMH induced groups. In comparison with DMH groups, EEAM (300 mg/kg) and 5-FU (20 mg/kg) treated animals shows significant ($P<0.001$) reduction in the total number of ACF, AC and crypts/ACF in total colon compared with distal and proximal colon (Table 2).

Regional Distribution of Aberrant Crypt Categories (1, 2, 3, 4 and = 5) in Rats Treated with DMH and EEAM: In comparison with DMH groups, the oral administration of EEAM (300 mg/kg) and 5-FU (20 mg/kg) was significantly reduced ($P<0.001$) the total number of ACF in total colon compared with distal and proximal colon (Table 3).

Apoptosis Index: The apoptosis index of Ethanolic extract of *Annona muricata* (300 mg/kg) is shown in Figure 1. The EEAM and 5-FU (20 mg/kg) was significantly ($P<0.05$), ($P<0.01$) increased apoptosis index respectively.

Haematological Parameters: Various haematological parameters are represented in Table 4. The administration of EEAM (300mg/kg) and 5-FU (20 mg/kg) were significantly ($P<0.001$) changes in RBC, WBC, HGB count when compared with DMH group.

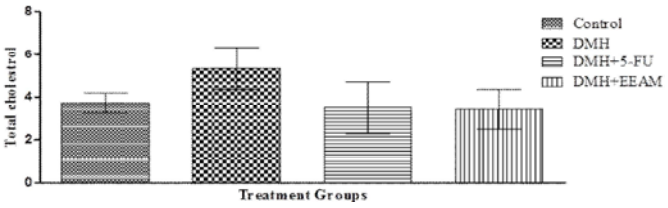


Fig. 1: Effect of Ethanolic extract of Annona muricata on Total cholesterol

Table 1: Body weight changes on treatment with Ethanolic extract of *Annona muricata* and DMH

Group	Initial body weight (gm)	Final body weight (gm)	Weight gain(gm)
Control	147.50±7.93	158.33±8.72	154.83±14.23
DMH	195.00±15.22	225.00±12.04	233.33±4.94
DMH+5-FU	166.66±9.88**	141.66±8.72***	181.66±11.37***
DMH+EEAM	138.33±12.22***	125.00±6.70***	129.16±4.54***

Values are Mean ±S.E.M, n=6 in each group.*P<0.05, ** P <0.01, *** P <0.001 when compared with disease control group (One way ANOVA fallowed by Dunnett's test).

Table 2: Effect of Ethanolic extract of *Annona muricata* on distribution of altered aberrant crypt foci (ACF) in proximal, distal and total of rats exposed to DMH and EEAM

Group	Total no. Of AC	Total no of ACF	Crypt/ACF
	Proximal colon		
Control	0	0	0
DMH	20.16±0.75	15.50±2.81	3.33±1.21
DMH+5-FU	18.66±0.81	12.00±1.09**	1.33±0.51***
DMH+EEAM	11.55±2.66***	9.83±1.60***	1.16±0.40**
	Distal colon		
Control	0	0	0
DMH	32.33±3.38	18.83±1.16	3.50±1.04
DMH+5-FU	26.66±2.16**	17.66±1.63	2.33±0.51**
DMH+EEAM	16.83±3.97***	11.50±1.22***	2.16±1.16**
Total Colon			
Control	0	0	0
DMH	33.66±2.25	26.16±1.60	3.66±0.81
DMH+5-FU	25.33±1.75***	23.33±1.21**	1.66±0.81***
DMH-+EEAM	21.16±1.47***	15.50±1.64***	1.50±0.83***

Values are Mean ±S.E.M, n=6 in each group.* P <0.05, ** P <0.01, *** P <0.001 when compared with disease control group (One way ANOVA fallowed by Dunnett's test).

Table 3: Regional distribution of aberrant crypt categories (1, 2, 3, 4 and = 5) in rats treated with DMH and EEAM.

	Number of aberrant crypts per ACF				
Group	1	2	3	4	≥5
	Proximal Colon				
Control	0	0	0	0	0
DMH	8.16±0.40	6.66±1.75	5.33±1.63	4.66±2.16	4.16±2.31
DMH+5-FU	6.33±1.36*	5.33±1.36	2.66±1.50**	2.16±1.47**	1.66±0.81**
DMH+EEAM	4.83±1.94***	3.50±1.37***	2.16±1.60**	2.00±0.63***	1.16±0.40**
	Distal Colon				
Control	0	0	0	0	0
DMH	22.66±1.03	21.50±1.04	19.50±1.87	16.83±1.32	14.33±1.21
DMH+5-FU	20.16±2.85*	20.83±0.68	15.33±0.81***	13.66±1.36**	13.66±1.03
DMH+EEAM	12.66±0.81***	12.66±0.75***	11.50±1.51***	10.33±2.58***	8.00±1.78***
	Total Colon				
Control	0	0	0	0	0
DMH	28.83±1.83	26.33±1.36	22.50±1.37	19.83±3.25	18.16±2.04
DMH+5-FU	27.50±2.07	15.33±2.16***	15.16±2.85***	13.66±2.16***	14.00±1.67***
DMH+EEAM	22.33±2.80***	15.16±2.85***	13.66±1.96***	12.50±1.64***	7.50±2.07***

Values are Mean ±S.E.M, n=6 in each group.* P <0.05, ** P <0.01, *** P <0.001 when compared with disease control group (One way ANOVA fallowed by Dunnett's test).

Table 4: Effect of Ethanolic extract of *Annona muricata* on various haematological parameters and Apoptosis index

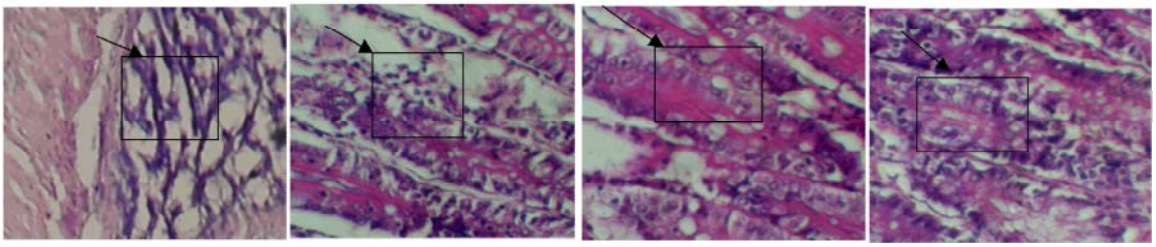
Group	RBC(cells/μL×10^3)	WBC(cells/μL×10^3)	HGB(g/dL)	PLT(cells/μL×10^3)	Apoptotic index
Control	6.33±0.49	11.50±0.3	11.16±0.54	181.83±18.78	3.16±1.60
DMH	7.50±0.61	14.60±0.90	18.50±0.76	150.16±15.64	1.16±0.40
DMH+5-FU	4.83±0.47**	6.20±0.40***	15.50±1.02*	165.66±17.45	3.33±0.81**
DMH+EEAM	4.50±0.42**	6.70±0.50***	16.66±1.11***	148.33±16.21	3.16±1.32*

Values are Mean ±S.E.M, n=6 in each group.* P <0.05, ** P <0.01, *** P <0.001 when compared with disease control group (One way ANOVA followed by Dunnett's test).

Table 5: Effect of Ethanolic extract of *Annona muricata* on relative weight of organs

	Relative Weight of individual organs (Wt. of organ/100g)							
Group	Liver	Kidney	Heart	Lungs	Pancreas	Spleen	Stomach	Colon
Control	15.01±0.39	0.85±0.02	0.62±0.13	1.49±0.21	0.72±0.04	0.48±0.02	1.25±0.02	1.14±0.02
DMH	16.33±0.32	1.14±0.03	0.81±0.03	1.75±0.04	0.82±0.06	0.51±0.03	1.62±0.06	1.34±0.02
DMH+5-FU	15.95±0.52***	1.44±0.24***	0.61±0.13***	1.53±0.14***	0.53±0.32	0.49±0.02**	1.42±0.42	1.31±0.08*
DMH+EEAM	7.5±0.15***	0.83±0.02***	0.82±0.06	1.37±0.14***	0.34±0.25	0.44±0.03***	1.38±0.39	1.31±0.06*

Values are Mean ±S.E.M, n=6 in each group.* P <0.05, ** P <0.01, *** P <0.001 when compared with disease control group (One way ANOVA followed by Dunnett's test).



(A) Control (B) DMH (C) 5-Florouracil (D) EEAM

(A) Rats treated with vehicle
(B) Rats treated with DMH alone
(C) Rats treated with 20 mg/kg of 5-Florouracil
(D) Rats treated with 300mg/kg of EEAM.

Fig. 2: Histopathology of Colon:

Relative Organs Weights: The administration of EEAM (300 mg/kg) and 5-FU (20 mg/kg) were significant (P<0.001) reduction in organ weights when compared with DMH group (Table 5).

Estimation of Cholesterol Levels: The EEAM (300 mg/kg) and 5-FU (20mg/kg) cholesterol level were significantly (P<0.01) decreased compared with DMH group (Figure 2).

Histopathology of Colon: Aberrant crypts (ACs) were identified as the crypts that were 2 to 3 times larger than the surrounding normal crypts. ACFs are early appearing lesions recognized on the colonic lumen surface in the treated rats. These ACFs are characterized by crypts with altered luminal epithelia and being larger than the adjacent normal crypts. There was observed the development of ACF and AC in the DMH treated rats. In comparison with DMH group, the administration of EEAM at a dose of 300 mg/kg body weight significantly reduced number of ACF and AC and administration of 5-FU also significantly reduced number of ACF and AC when compared to DMH induced group respectively.

DISCUSSION

Colon cancer is one of the most common malignancies in today. DMH is used to produce colon cancer, which is metabolized into the carcinogenic metabolite without previous metabolism by other tissues or colon bacteria in rat. The ultimate carcinogenic metabolite of DMH is responsible for methylation of the DNA bases of various organs, including epithelial cells in the proliferative compartment of the crypts, which results in a great loss of colonic cells by apoptosis, an increase in proliferation [17] and an apparent increase in mutations of colonic epithelial cells. All these alterations are likely to produce increase in inflammatory growth factors such as TNF α, TGF β and oxidative stress [18]. There was also an

increase in mortality and decrease in body weight of the animals treated with DMH, which may be due to induction of cancer by DMH. In the present study rats treated with DMH were found to show presence of ACF which indicates development of carcinomatous changes. Preneoplastic lesions such as ACF, which occurs in 30 days after DMH administration or even earlier, have been extensively, used as endpoint in short term carcinogenesis and chemo preventive studies.

In our present study, DMH treatment led to increase in the grossly visible aberrant crypt foci (ACF) after a period of 30 days. In the study increased crypt size was observed in DMH treated rats and moreover at the end of the 30 days study the percentage of ACF in rats treated with DMH alone w as increased than in treated group. It also observed the change in colon crypt pathology and change in apoptosis [19]. A large numbers of agents including natural and synthetic compounds have been identified to possess potential cancer chemo preventive value, inhibiting mutagenesis, hyper proliferation or induce apoptosis or differentiation, which are critical characteristics of chemoprevention.

The chemo preventive effects of *Annona muricata* leaves might be attributed to the presence of a class of compound obtained from Annona species, acetogenins. It has been reported that the main antitumorous compound, annonacin was effective against various *in vitro* cancer cell lines as well as *in vivo* lung cancer. Nowadays, even without any scientific validation, many cancer patients and health practitioners are adding the natural leaf and stem of *Annona muricata* (with over 40 documented naturally-occurring acetogenins including annonacin) as a complementary therapy to their cancer protocols. After all, *Annona muricata* has a long history of safe use as a herbal remedy for other conditions for many years and research indicates that the anti-tumorous acetogenins are selectively toxic to just cancer cells and not in healthy cells and miniscule amounts. Weight loss is an important prognostic factor in cancer; the higher the extent of weight loss, the shorter the survival time. The prognostic effect of weight loss is greatest in patients with a more favourable prognosis. Treatment with EEAM reduces weight gain and DMH induced mortality in animals showed good prognosis in colon cancers. The results of the present study demonstrated that administration of ethonolic extract of *Anonna muricata* at a dose of 300 mg/kg body weight significantly inhibited DMH-induced colon carcinogenesis in rats.The finding indicate *Anonna muricata* is significantly (P<0.001) reduced the number of ACF development in the colon, weight of the organs, heamatological parameters and improvement in histopathological changes and weight gain. It is also significantly (P<0.05) induced apoptosis and biochemical parameter cholestrool level decreased significantly(P<0.01) when compared with DMH groups.Thus the data strongly suggest that the colon cancer inhibitory effect observed with ethanolic extract of the *Anonna muricata* leaves at the dose of 300 mg/kg body weight.

CONCLUSION

Based on the above results it has shows that Ethanolic extract of *Annona muricata* has anti cancer property. Ethanolic extract of *Annona muricata* administration at a dose of 300 mg/kg reduced the formation of ACF and number of aberrant crypts. It also increased apoptosis index and increased weight gain and improved histopathological changes. Based on this Ethanolic extract of *Annona muricata* having anti cancer properties at a dose of 300 mg/kg body weight.

ACKNOWLEDGEMENT

The authors are grateful to Secretary, Viswambhara Educational Society and Warangal for providing the necessary facilities to carry out the study.

REFERENCES

1. Boyle, P. and B. Levin, 2008. World Cancer Report 2008, International Agency for Research on Cancer Press, Lyon, 42:511.
2. Scott, M.P., 2003. A twist in the Hedgehog's tale. Nature, 425(6960): 780-782.
3. Folkman, J. and R. Kalluri, 2004. Cancer without disease. Nature, 427(6977): 787.
4. Devasena, T., K.N. Rajasekaran, G. Gunasekaran, P. Viswanathan and V.P. Menon, 2003. Anticarcinogenic effect of bis-1,7-(2-hydroxyphenyl)-hepta-1,6-diene-3,5-dione a curcumin analog on DMH-induced colon cancer models. Pharmacological. Resarch, 47: 133-140.
5. Salim, E.I., A.E. Abou-Shafey, A.A. Masoud and S.A. Elgendy, 2011. Cancer Chemopreventive Potential of the Egyptian Flaxseed Oil in a Rat Colon Carcinogenesis Bioassay-Implications for its Mechanism of Action. Asian Pacific Journal of Cancer Prevention, 12(9): 2385-2392.

6. Heinrich, M., M. Kuhnt, C.W. Wright, H. Rimpler, J.D. Phillipson, A. Schandelmaier and D.C. Warhurst, 1992. Parasitological and microbiological evaluation of mixe Indian medicinal plants (Mexico). Journal of Ethnopharmacology, 136(1): 81-85.
7. Ilan, A., K. Sarah, N. Inna, R. Ehud and S. Shiran, 2010. Chemopreventive effect of coltect, a noval dietary supplement, alone and in combination with 5-aminosalicylic acid in 1,2-dimethylhydrazine-induced colon cancer in rats. Therapeutic advances in gastroentrology. Therapeutic Advances in Gastroenterology, 3(5): 281-289.
8. Ajit, B.P. and S. Asha, 2013. *In-vivo* and *in-vitro* models on colon cancer. International Journal of Pharmacy and Biological Sciences, 3(1): 24-31.
9. Ecobichon, D.J., 1997. Fixed Dose Procedure Guideline 420. The basis of Toxicity testing. New York: CRC Press, pp: 2.
10. Mecklin, J.P., L.B. Svendsen, P. Peltomäki and H.F. Vasen, 1994. Hereditary nonpolyposis colorectal cancer. Scandinavian Journal of Gastroenterology, 29(8): 673-677.
11. Hamiza, O.O., M.U. Rehman, M. Tahir, R. Khan, A.Q. Khan, A. Lateef, F. Ali and S. Sultana, 1994. Amelioration of 1,2 Dimethylhydrazine (DMH) Induced Colon Oxidative Stress, Inflammation and Tumor Promotion Response by Tannic Acid in Wistar Rats. Asian Pacific Journal of Cancer Prevention, 13(9): 4393-4402.
12. De Sousa, O.V., G.D. Vieira, R.G. de Jesus, J. De Pinho, C.H. Yamamoto and M.S. Alves, 2010. Antinociceptive and Anti-Inflammatory Activities of the Ethanol Extract of *Annona muricata* L. Leaves in Animal Models. International Journal of Molecular Sciences, 11(5): 2067-2078.
13. Pretlow, T.P., B.J. Barrow, W.S. Ashton, M.A. O'Riordan, T.G. Pretlow, J.A. Jurcisek and T.A. Stellato, 1991. Aberrant crypts: putative preneoplastic foci in human colonic mucosa. Cancer Resarch, 51(5): 1564-1567.
14. Roshni, P.S., M. Dhaval and K.G. Ramesh, 2014. Anti cancer activity of colon specific osmotic drug delivery of acetone extract of Quercus Infectoria Olivier, Fagaceae in 1,2-dimethylhydrazine-induced colon cancer in rats. Molecular Cytogenetics, 7(Suppl 1): 14.
15. Roncucci, L., D. Stamp, A. Medline, J.B. Cullen and W.R. Bruce, 1991. Identification and quantification of aberrant crypt foci and microadenomas in the human colon. Human Pathology, 22(3): 287-294.
16. Sofowra, A., 1993. Medicinal Plants And traditional Medicine In Africa. Spectrum Books Ltd. Ibadan, Nigeria, pp: 191-289
17. Shani, J. and P.V. Danenberg, 1984. Evidence that intracellular synthesis of 5-fluorouridine-5'-phosphate from 5-fluorouracil and 5-fluorouridine is compartmentalized. Biochem and Biophysical Research Communication, 122(1): 439-445.
18. Hamizah, S., A.H. Roslida, O. Fezah, K.L. Tan, Y.S. Tor and C.I. Tan, 2012. Chemopreventive Potential of *Annona Muricata* L Leaves on Chemically-Induced Skin Papillomagenesis in Mice. Asian Pacific Journal of Cancer Prevention, 13(6): 2533-2539.
19. Volate, S.R., D.M. Davenport, S.J. Muga and M.J. Wargovich, 2005. Modulation of aberrant crypt foci and apoptosis by dietary herbal supplements (quercetin, curcumin, silymarin, ginseng and rutin). Carcinogenesis, 26(8):1450-1456.