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Original Article

Amelioration of 1, 2 Dimethylhydrazine Induced Tumor Promotion Response by Novel Benzimidazole Derivative Nanoparticle in Wistar Rats

S.S. Rajendran[1*], G. Geetha[2], R. Venkatanarayanan[1], N. Santhi[1]
[1]Department of Pharmaceutical Chemistry, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore, Tamil Nadu - 641 402, INDIA.
[2]Department of Pharmaceutical Chemistry, PPG College of Pharmacy, Saravanampatti, Coimbatore, Tamilnadu-641 035, INDIA.

ABSTRACT

Background: Colon cancer is one of the most common malignancies in many regions of the world and is thought to arise from the accumulation of mutations in a single epithelial cell of the colon and rectum. The benzimidazole comprises a important pharmacophore and privileged structure in modern drug discovery. Various substituted benzimidazole derivatives have been found to possess potential anticancer properties. **Objective:** The study aimed to prove the anti-colon cancer activity of novel benzimidazole derivative 4-(1H-benzo[d]imidazol-2-yl)-6-phenylpyrimidin-2-amine loaded chitosan nanoparticle (BZI 3 nano) by an 1, 2 Dimethylhydrazine (DMH) Induced rat model *in-vivo* study and identify the targeting efficiency of BZI 3 nano to treat colorectal cancer. **Method:** The effect of novel benzimidazole derivative 4-(1H-benzo[d]imidazol-2-yl)-6-phenylpyrimidin-2-amine loaded chitosan nanoparticle (BZI 3 nano) on the formation of aberrant crypt foci (ACF), apoptosis, histopathology, body weight, organs weight and heamotological parameters were studied in 1,2-dimethylhydrazine (DMH)-induced colon cancer in rats. **Results:** BZI 3 nano (5 mg/kg, *p.o*) administration significantly reduced ACF number and increased the weight gain and apoptotic index compared to DMH treated group. The histological alterations induced by DMH were also significantly improved. **Conclusion:** *In-vivo* anticancer activities results revealed that the presence substituted benzimidazole derivative nanoparticle (BZI 3 nano) could have the anticancer potential of the scaffold and selective, good target for drug discovery, which can be regarded as promising anticancer potential.

Key words: Benzimidazole derivative, Nanoparticles, DMH, ACF.

Correspondence

S.S. Rajendran, Assistant Professor, Department of Pharmaceutical Chemistry, RVS College of Pharmaceutical Sciences, Coimbatore, Tamil Nadu - 641402, INDIA.

Phone: +919894897258

Email: researchrajendran@gmail.com

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INTRODUCTION

Cancers are the second cause of death and have the highest burden of diseases in 21st century. 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 or may develops into a lethal tumor or cancer. 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.[1] Colon cancer is the second leading cause of cancer related deaths in USA.[2] Recently colon cancer incidence has been increasing in India year by year.[3] The efficacy of present cancer chemotherapy is mainly limited by the toxicity associated with the anticancer drugs to normal tissues. This limitations result from the fact that anticancer drugs presently used in chemotherapy lack efficient selectivity towards tumor cells. This necessitates the development of a novel nanoparticle delivery system to overcome these current obstacles in convention drug therapy.[4]

Benzimidazole derivatives are comprises a relatively huge, growing, key pharmacophore and privileged structure in modern drug discovery.[5] Now a days is a moiety of choice which has numerous pharmacological properties. Substituted benzimidazole derivatives have found applications in diverse therapeutical areas.[6] Principally 2-substituted benzimidazole derivatives have been found to possess potential anticancer properties.[7]

A rational approach for design the next generation inhibitors, benzimidazole derivatives,[8] The novel benzimidazole derivative 4-(1H-benzo[d] imidazol-2-yl)-6-phenylpyrimidin-2-amine loaded chitosan nanoparticles formulated and characterized, these results demonstrate that the possibility of delivering synthesised novel benzimidazole derivative 4-(1H-benzo[d]imidazol-2-yl)-6-phenylpyrimidin-2-amine loaded nanoparticle (BZI 3 nano) to colorectum with enhanced encapsulation efficiency. Additionally the nanoparticle (BZI 3 nano) have been evaluated for *In vitro* cytotoxicity in Caco2 and MCF- 7 cell lines. *In vitro* cytotoxicity study suggested the safety of the prepared novel nanoparticle (BZI 3 nano), which can be potential carrier to deliver hydrophilic drugs to target colorectum.[9] Further *In vivo* will confirm the targeting efficiency of nanoparticle (BZI 3 nano) to treat colorectal cancer.

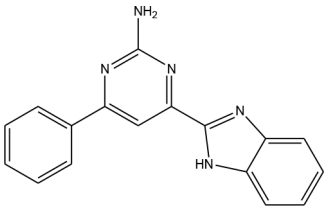
Hence the present work was undertaken to scientifically prove the anti-colon cancer activity of novel benzimidazole derivative 4-(1H-benzo[d] imidazol-2-yl)-6-phenylpyrimidin-2-amine loaded chitosan nanoparticle (BZI 3 nano) by an *in-vivo* study.

MATERIALS AND METHODS

Reagents and Chemicals

1, 2-Dimethyl hydrazine (DMH) were purchased from Sigma Chemicals private limited, Bangalore, Methylene blue from Sisco Research Laboratories private limited, Mumbai, Haematoxylin from Merck Specialties private limited, Mumbai. All other chemicals and reagents used were of analytical grade.

Drug Profile[9-10]



4-(1H-benzo[d]imidazol-2-yl)-6-phenylpyrimidin-2-amine

Molecular Formula: $C_{17}H_{13}N_5$: Molecular Weight: 287.32

Mp: 146 – 149 ^{0}C: R*f* : 0.75

The 2- substituted novel benzimidazole derivative were synthesised by using incorporated with heterocycle like pyrimidine and backbone of chalcones. The synthesised compound were screened for their *in vitro* anticancer activities against the CaCo-2 and MCF-7 cell lines. The 24 hours Caspase study revealed that compound 4-(1H-benzo[d]imidazol-2-yl)-6-phenylpyrimidin-2-amine showed 2 fold activities in caspase 3 and 9 pathway, single fold activity in caspase 8 pathways. The novel benzimidazole derivative 4-(1H-benzo[d]imidazol-2-yl)-6-phenylpyrimidin-2-amine loaded chitosan nanoparticles formulated and characterized. These results revealed that the presence 2-substituted benzimidazole derivative could have the anticancer potential of the scaffold.

Animals [11-13]

In this study, A total of 24 Male Wistar albino rats (150-180 gm) were selected and procured from RVS College Of Pharmaceutical Sciences, Tamilnadu, India. All animals were maintained under environmentally controlled conditions of 25±2° C, relative humidity of 45 to 55 % and 12 h light-12 h 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 (IAE1012/c/16/CPCSEA – Corres - 2013).

Acute Toxicity Study [14-15]

Acute toxicity studies and fixing of dose for the newly formulated benzimidazole derivative nanoparticle (BZI 3 nano). Acute oral toxicity test was carried out according to the OECD guideline No. 423.Male *Wistar* Albino Rats were kept for overnight fasting prior to drug administration. The sighting study of the compound was carried out on the group (3 numbers) of albino rats of *wistar* strain of male sex fixed dose method. 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.

Preparation of DMH

DMH was dissolved in 1 mM EDTA just prior to use and the pH adjusted to 6.5 with 1 mM NaOH to ensure the stability of the carcinogen.

Induction of colon cancer

The rats were given subcutaneous injections of DMH twice a week for 2 weeks at a dose of 30 mg/kg body weight.

Preparation of standard drug (5-Fluorouracil)

The 5-Fluorouracil was dissolved in normal saline

Preparation of test drug (BZI 3 nano)

BZI 3 nano was suspended in 5% gum acacia and each rat received a daily 1 ml as suspension at a dose of 5 mg/kg body weight orally by oral gavage throughout the experimental period.

Experimental Design and Treatment Schedule[16]

Group I served as normal control, which received vehicle (gum acacia suspension), Group II rats served as disease control were given subcutaneous injections of DMH twice a week for 2 consecutive weeks at 30 mg/kg b.w., in addition to this Group III rats were given standard (5-Fluorouracil) at a dose of 20 mg/kg body weight daily by *i.p* and Group IV rats were given formulated novel benzimidazole derivative nanoparticle (BZI 3 nano) at a dose of 5 mg/kg body weight daily for total 30 days of study period by oral route.

Group I : Vehicle control: received 1ml of normal saline (*p.o*)

Group II : Disease control (DMH: 30 mg/Kg, *s.c*)

Group III: Disease + 5-Fluorouracil (5-FU: 20 mg/kg, *i.p*)

Group IV: Disease + BZI 3 nano (5 mg/kg, *p.o*)

In vivo Methods

Body Weight Changes[17-18]

The body weight changes of the Control, DMH, 5-FU and BZI 3 nano 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.

Determination of Aberrant Crypt Foci (ACF)[16]

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 was 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.

Apoptosis Measurement in Colonic Mucosa[19]

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 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 (AI).

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

Haematological Evaluation[20]

Before 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.

Individual Organs Weights[20]

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.

Histopathology of Colon[21]

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 h and after fixation, the specimens were dehydrated in ascending grades of ethanol, cleared in benzene and embedded in paraffin wax. Blocks were made and 5µm 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 histo architecture of colonic mucosa.

Statistical Analysis

Results were given as means ± SD of each group. Data were analyzed by one-way analysis of variance and any significant difference among treatment groups was evaluated by Dunnet's Test. The results were considered statistically significant at $P < 0.05$.

RESULTS

Acute Toxicity Study

Acute toxicity studies and fixing of dose for the newlyl formulated benzimidazole derivative nanoparticle (BZI 3 nano). Acute oral toxicity test was carried out according to the OECD guideline No. 423.Male *Wistar* Albino Rats were kept for overnight fasting prior to drug administration. The sighting study of the compound was carried out on the group (3 numbers) of albino rats of *wistar* strain of male sex following fixed dose method. Only one animal among the group of three was tested with initial dose 5mg/kg via orally administration and observe once in 30 min for the first 4 h and periodically during the first 24 h for event toxicity or mortality. Since there was no such toxicity or mortality, the other animals in the group were testes with initial test dose 5mg/kg and observed for 14 days, the test compound did not produce any signs of toxicity or mortality. The procedure of sighting study was repeated at a higher dose of 25mg/kg and observed for 14 days, and the test compounds did not produce any signs of toxicity or mortality. At 50mg/kg sighting study, the compound produced mortality in more than 40% of the population with tremors and convulsion. Hence, 5mg/kg was fixed the dose strength for the main study.

In vivo Methods

Body Weight Changes

During the experimental period 30 days, the carcinogen-exposed rats (Group II- DMH treated) exhibited a significantly ($p< 0.05$) low gain in body weight (Figure 1) and a low growth rate throughout the experimental period as compared to Groups III (DMH+5FU) and IV (DMH+ BZI 3 nano) Oral administration of BZI 3 nano at a dose of 5 mg/kg b.w resulted in a significant improvement in weight gain relative to treatment with DMH alone. (Table 1)

Determination of Aberrant Crypt Foci (ACF)

ACF formation was observed in all DMH induced groups. The majority of ACF appeared in the distal colon of the rats injected with DMH. Oral administration of BZI 3 nano at 5 mg/kg b.w. inhibited the formation as well as the total number of ACF (Table 2 and Figure 2), as compared to rats injected with DMH alone. No ACF formation was observed in the control (group I - Control).

Table 1: Body weight changes on treatment with DMH, 5FU and BZI 3 nano.

Group/Treatment	Initial body weight(g)	Final body weight(g)	Weight gain(g)
Control	165.16 ± 8.93	216.50 ± 7.04	51.34 ± 7.92
DMH	162.50 ± 7.72	191.83 ± 8.72	29.33 ± 5.24
DMH+5FU	173.33 ± 7.93	217.60 ± 6.54	44.27 ± 3.04 ***
DMH+ BZI 3 nano	163.50 ± 6.54	203.33 ± 6.70	39.83 ± 4.54 **

Values are mean ± SD, n=6 in each group, statistically significant***p<0.001 **p<0.01,*p<0.05 when compared with disease control (DMH treated) group (One way ANOVA followed by Dunnet's test).

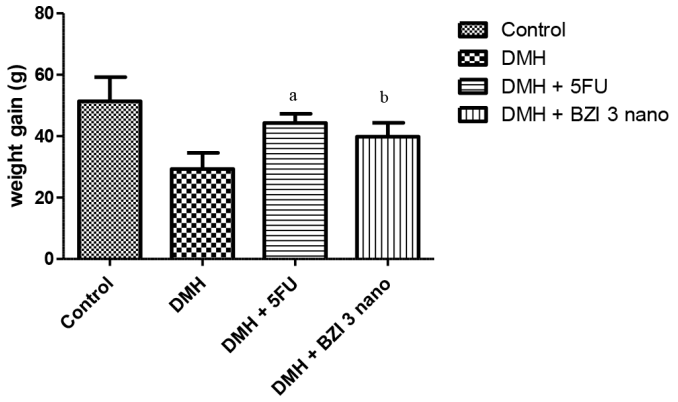


Figure 1: Body weight changes on treatment with DMH, 5FU and BZI 3 nano. Values are mean ± SD, n=6 in each group, statistically significant a***p<0.001, b**p<0.01 when compared with disease control (DMH treated) group (One way ANOVA followed by Dunnet's test).

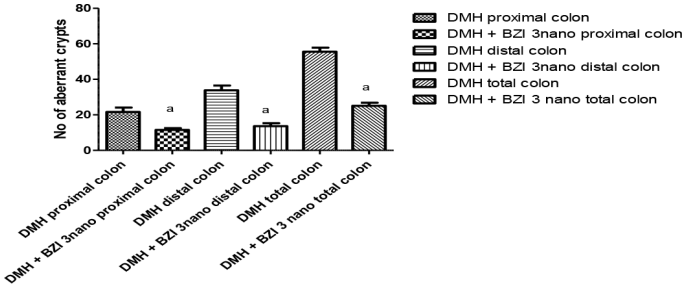


Figure 2: Aberrant crypt foci scoring
Values are mean ± SD, n=6 in each group, statistically significant a***p<0.001, when compared with disease control (DMH treated) group (One way ANOVA followed by Dunnet's test).

Regional distribution of aberrant crypt categories (1, 2, 3, 4 and ≥ 5) in rats treated with DMH, 5FU and BZI 3 nano.

ACF formation was observed in all DMH induced groups. The majority of ACF appeared in the distal colon of the rats injected with DMH. Oral administration of BZI 3 nano at 5 mg/kg b.w. inhibited the formation as well as the total number of ACF, AC (Table 3 and Figure 3) as compared to rats injected with DMH alone. No ACF formation was observed in the control (Group I -control).

Apoptosis Measurement in Colonic Mucosa

The apoptosis index (AI %) was estimated as the percentage of apoptotic cells (i.e., with cellular retraction and condensation, condensed or fragmented nuclear chromatin and formation of apoptotic bodies) among

Table 2: Aberrant crypt foci scoring - Distribution of altered aberrant crypt foci (ACF) category in proximal, distal and total colon of rats exposed to DMH, 5FU and BZI 3 nano.

Group/Treatment	No. of animals	Total no. of AC	Total no. of ACF	Crypt/ACF
		Proximal colon		
Control	6	0	0	0
DMH	6	21.66 ± 2.47	16.16 ± 3.41	1.34 ±0.70
DMH+5FU	6	15.33 ± 3.26	10.50 ± 1.19	1.46 ±0.81
DMH+ BZI 3 nano	6	11.50 ± 1.02	9.33 ± 1.77	1.23 ±0.83
		Distal colon		
Control	6	0	0	0
DMH	6	33.83 ± 2.68	19.16 ± 2.66	1.76 ±0.51
DMH+5FU	6	24.16 ± 1.68	15.66 ± 3.27	1.54±0.40
DMH+ BZI 3 nano	6	13.60 ± 1.72	9.50 ± 1.34	1.43±0.72
		Total colon		
Contro	6	0	0	0
DMH	6	55.59 ±2.25	35.32±1.64	1.57±0.83
DMH+5FU	6	39.49 ±1.47	26.16±1.21	1.50±0.81
DMH+ BZI 3 nano	6	25.10±1.75***	18.83±1.64***	1.33±0.80***

Values are mean ± SD, n=6 in each group, statistically significant***p<0.001 **p<0.01,*p<0.05 when compared with disease control (DMH treated) group (One way ANOVA followed by Dunnet's test).

Table 3: Regional distribution of aberrant crypt categories (1, 2, 3, 4 and ≥ 5) in rats treated with DMH, 5FU and BZI 3 nano.

Group/Treatment	No. of animals	Number of aberrant crypts per ACF				
		1	2	3	4	≥5
		Proximal colon				
Control	6	0	0	0	0	0
DMH	6	8.33 ± 3.61	6.83 ±2.26	3.16 ±1.72	2.50 ±1.86	2.33 ±1.37
DMH+5FU	6	5.83 ± 2.33	4.33 ±3.17	2.83 ±1.68	1.83 ±1.23	1.66 ±0.52
DMH+ BZI 3 nano	6	3.83 ± 1.67**	2.83 ±0.17	2.16 ±1.32	1.33 ±0.87	1.16 ±0.89
		Distal colon				
Control	6	0	0	0	0	0
DMH	6	15.66 ± 3.77	16.50 ±5.62	12.16 ±1.06	10.83 ±1.67	8.16 ±0.63
DMH+5FU	6	10.16 ± 4.27	14.33 ±3.67	8.50 ±4.4	8.33 ±2.81	7.33 ±4.5
DMH+ BZI 3 nano	6	8.16 ± 2.82**	10.16 ±2.12**	5.16 ±2.89	5.33 ±2.16	5.16 ±1.80*
		Total colon				
Control	6	0	0	0	0	0
DMH	6	23.99 ±1.82	23.33±1.34	15.32±1.38	13.33±3.20	10.49±2.04
DMH+5FU	6	15.99 ±2.06	18.66±2.16	11.33±2.85	10.16±2.16	8.99±1.67
DMH+ BZI 3 nano	6	11.99±2.50 **	12.99±2.80**	7.32±1.90	6.66±1.64	6.32±2.07

Values are mean±SD, n=6 in each group, statistically significant a **p<0.01, b*p<0.05when compared with disease control (DMH treated) group (One way ANOVA followed by Dunnet's test).

the total number of counted cells in a whole colonic crypt. The apoptotic index was increased in group treated with DMH+ BZI 3 nano compared to DMH treated group and DMH + 5FU group. (Table 4 and Figure 4).

Haematological Evaluation

Various haematological parameters are compared among the groups before and after induction of colon cancer as shown in Table 5.

Individual Organs Weights

Relative weights of various organs are compared among the groups after induction of colon cancer as shown in Table 6.

Histopathology of Colon

Pathology of haematoxylin and eosin stained sections of colon slides showed that there was a wide range of histology from minor atypia to

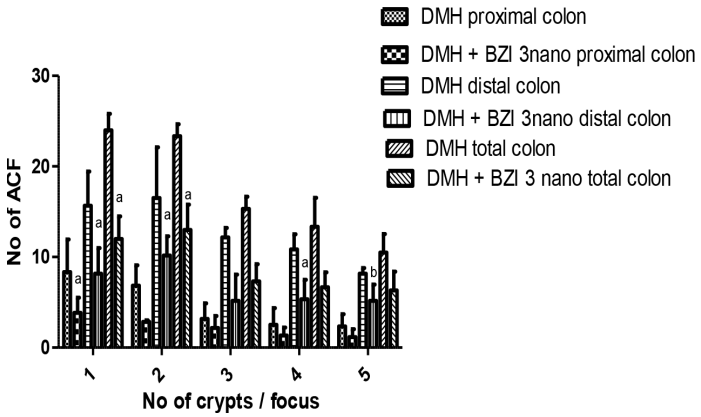


Figure 3: Regional distribution of aberrant crypt categories

severe dysplasia in DMH treated group than BZI 3 nano treated group. There was no change in colonic mucosa of control group rats (Figure 5).

DISCUSSION

In an effort to establish novel benzimidazole derivative with anticancer activity and achieve the colon target , we performed *In vivo* method to confirm the targeting efficiency of synthesised novel benzimidazole derivative 4-(1H-benzo[d]imidazol-2-yl)-6-phenylpyrimidin-2-amine loaded chitosan nanoparticle (BZI 3 nano) to treat colorectal cancer.

Acute toxicity studies performed on newly formulated benzimidazole derivative nanoparticle (BZI 3 nano) (according to OECD guidelines 423). At 50mg/kg sighting study, the compound produced mortality in more than 40% of the population with tremors and convulsion. Hence, from this 1/10th of MTD was selected and the effective doses were fixed as 5mg/kg dose strength for the further pharmacological studies.[15]

In vivo study we used DMH as colon cancer inducer and synthesized novel benzimidazole derivative nano particle (BZI 3 nano) as the test drug.

Weight loss reflects a common feature of gastrointestinal tumours, and weight loss is a frequent cause of concern, the aggressiveness of the disease. As expected carcinogen (DMH) treated animals in our study showed decreased weight gain compared to other groups. It may be due to reduced food intake. In addition to increased hepatic gluconeogenesis and altered glucose metabolism reduces the energy sources leading to a significant weight loss in DMH treated animals.[18]

Table 4: Effect of DMH, 5FU and BZI 3 nano on apoptotic indexes.

Group/Treatment	Apoptotic index
Control	4.16
DMH	0.80
DMH+5FU	3.33 **
DMH+ BZI 3 nano	3.83 **

Values are mean ±SD, n=6 in each group, statistically significant **p<0.01when compared with disease control (DMH treated) group (One way ANOVA followed by Dunnet's test).

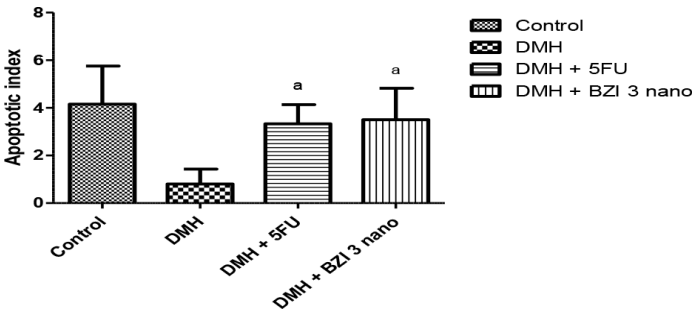


Figure 4: Effect of DMH, 5FU and BZI 3 nano on apoptotic indexes. Values are mean ±SD, n=6 in each group, statistically significant a**p<0.01, when compared with disease control (DMH treated) group (One way ANOVA followed by Dunnet's test).

In this study, colons were examined for ACF 30days after the first injection of DMH. In our present study increased crypt number was observed in proximal, distal and total colons of DMH-treated rats than in treatment group. We also identified that the inhibitory effect of BZI 3 nano on the development of ACF was more pronounced in the entire period treatment regimen as compared to the other treatment groups. Data from ACF incidence indicated that all rats treated with DMH developed ACF. The results demonstrated that administration of BZI 3 nano suppress DMH-induced ACF development. BZI 3 nano did not affect the number of crypts per ACF but actually suppressed the total number of ACF. Besides, the decrease in numbers of ACF suggests that BZI 3 nano may inhibit the growth of ACF. The no of ACF in the distal colon were more than the proximal colon.[19]

Table 5: Effect of DMH, 5FU and BZI 3 nano on various haematological parameters.

Hemotological parameters	Change in hemotological parameters after induction							
	Control		DMH		DMH +5FU		DMH + BZI 3 nano	
	Before	After	Before	After	Before	After	Before	After
WBC (cells/μL×10³)	11.16±1.8	11.33±2.4	11.50±1.90	14.83±2.40	11.83±2.47	8.66±3.50**	11.66±3.45	9.50±4.20**
RBC (cells/μL×10⁶)	6.83±0.06	7.50±0.26	6.50±1.09	5.66±1.20	6.16±1.40	4.66±0.80**	6.50±1.05	4.83±0.0**
HGB(g/dL)	11.83±1.08	12.16±1.04	11.33±2.32	9.50±2.56	11.83±2.02	7.50±1.08*	11.16±2.50	9.83±1.40**
PLT (cells/μL×10³)	185.83±18.78	187.33±19.06	185.50±15.64	172.16±1.45	185.16±15.45	167.66±16.50	185.83±16.21	170.16±15.40
HCT (%)	30.16±1.21	32.50±4.09	30.66±3.50	27.83±4.65	30.50±5.80	22.50±4.20	30.83±5.40	28.66±4.65

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 6: Effect of DMH, 5FU and BZI 3 nano on relative weight of organs.

Relative wt of organs (wt of organ/100g)	Group/Treatment			
	Control	DMH	DMH+5FU	DMH + BZI 3 nano
Liver	16.85±0.07	12.29±0.4	14.61±0.06**	13.50±0.18**
Kidneys	0.93±0.04	0.52±0.02	0.7±0.01**	0.66±0.02**
Heart	0.49±0.03	0.23±0.02	0.39±0.01	0.32±0.01
Lungs	0.85±0.01	0.51±0.01	0.74±0.01**	0.69±0.01***
Pancreas	0.81±0.02	0.57±0.01	0.78±0.005	0.71±0.02
Spleen	0.39±0.01	0.19±0.01	0.35±0.01**	0.31±0.01***
Colon	0.78±0.01	0.56±0.02	0.7±0.01	0.64±0.05
Stomach	0.80±0.01	0.48±0.01	0.72±0.01	0.65±0.03

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).

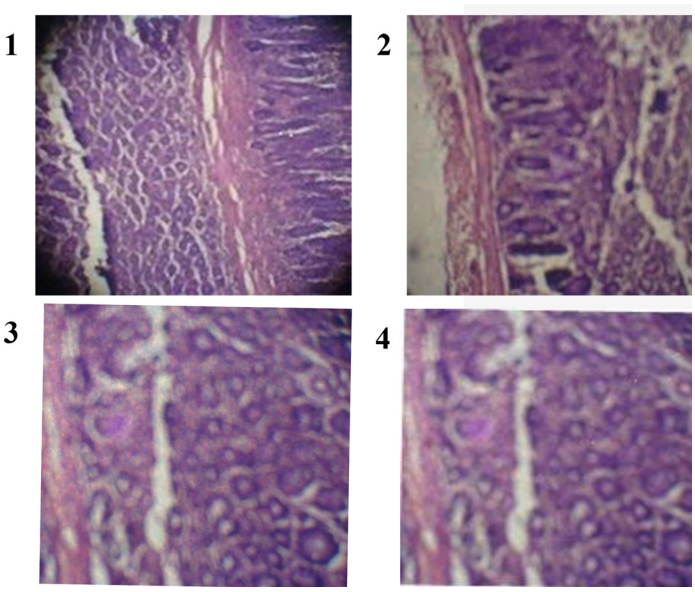


Figure 5: Histopathology of Colon.
(1) Control, (2) DMH Treated, (3) DMH + 5 FU, (4) DMH + BZI 3 nano
(1) Topographical view of normal crypt (40×). (2) Histological changes in the colonic mucosa on DMH administration shows thickened mucosa with densely packed inflammatory cell infiltration and a higher degree of hyperplasia (40×). (3) Represents 20 mg/kg b.w., 5FU supplemented rat colon showing mucosal thickening and scattered or no infiltration of inflammatory cells in the mucosal layer (40×). (4) Represents 5 mg/kg b.w., BZI 3 nano supplemented rat colon showing mucosal thickening in few areas and scattered or no infiltration of inflammatory cells in the mucosal layer (40×).

Pathology of haematoxylin and eosin stained sections of colon slides revealed that there was a broad range of histology from minor atypia to severe dysplasia in DMH treated group than BZI 3 nano treated group. There was no change in colonic mucosa of control group rats.[20]

Besides the reduction of ACF, based on the results it also revealed that BZI 3 nano induces apoptosis the apoptotic index was high in rats treated with DMH alone.

The results of the present study showed that administration of BZI 3 nano at a dose of 5mg/kg body weight during either the initiation, post-initiation or entire period phase significantly inhibited DMH-induced colon carcinogenesis in rats. Our findings indicate that BZI 3 nano is significantly reduced the number of ACF development in the colon. Several studies have suggested the growth features of ACF and dysplastic ACF and their location as a measure of the biological efficacy of the modifiers of colon carcinogenesis. Thus the data strongly suggest that the colon cancer inhibitory effects observed with BZI 3 nano at a dose of 5 mg/kg body weight. The inhibitory effect of BZI 3 nano may also due to the induction of apoptosis.

CONCLUSION

In vivo cytotoxicity study suggested the safety of the prepared synthesized novel benzimidazole derivative 4-(1H-benzo[d]imidazol-2-yl)-6-phenylpyrimidin-2-amine loaded chitosan nanoparticle (BZI 3 nano) which can be potential carrier to deliver hydrophilic drugs to target colorectum.

Benzimidazole derivative nanoparticle explored to benefit modern pharmacotherapy and drug discovery. Since only limited scientific studies have been carried out so far, a great potential exists to probe the potential therapeutic benefit from the benzimidazole derivatives nanoparticles used in cancer therapy. For reaping such benefit, improving future synthetic research in the cancer therapy is essential.

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CONFLICT OF INTEREST

All authors have none to declare.

ABBREVIATIONS

ACF: Aberrant Crypt Foci, **EDTA:** Ethylene Diamine Tetra Acetic Acid, **DMH:** 1, 2 Dimethylhydrazine, **BZI 3:** Benzimidazole derivative, **BZI 3 nano:** Benzimidazole derivative nanoparticle, **FU:** 5-Fluorouracil.

SUMMARY

Nanotechnology, which are being engineered for the targeted delivery of anticancer drugs. Traditional anticancer agents have a drawback that they need an active transport mechanism to penetrate the cells. This is a major drawback to reach adequate concentration of drug inside the cells. To overcome this disadvantage and developed a novel anticancer agent as benzimidazole derivative nano particle, that could be selective and good target for drug discovery. Used nanotechnology for construct the synthesized benzimidazole derivative as nano particle for provide the customizable, targeted drug delivery vehicles capable of ferrying large

doses of chemotherapeutic agents or therapeutic genes into malignant cells while sparing healthy cells. in-vivo anticancer activity of novel benzimidazole nanoparticle (BZI 3 nano) results revealed that the presence substituted benzimidazole derivative nanoparticle could have the anticancer potential of the scaffold and selective, good target for drug discovery, which can be regarded as promising anticancer potential.

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