Acta Biomedica Scientia

e - ISSN - 2348 - 2168
Print ISSN - 2348 - 215X

www.mcmed.us/journal/abs

Research Article

EVALUATION OF ANTICANCER ACTIVITY OF *COLDENIA PROCUMBENS* LINN IN 1, 2-DIMETHYL HYDRAZINE INDUCED COLON CANCER ON RAT MODEL

K.Ramachandran* and R.Venketnarayanan[1]

*Lecturer & Hospital Pharmacy Incharge, Department of Pharmacy, College of Medical Sciences, Bharatpur 10, Chitwan District, Postal code -44200, Nepal.
[1]Department of Pharmacology, R. V. S. College of Pharmaceuticals Sciences, Coimbatore, Tamilnadu, India – 641402.

ABSTRACT

Background: Cancer is one of the significant health issues in the developed and developing nations. Anticancer activity is to prevent or suppress or reverse the carcinogenic progression by natural and synthetic or biological and chemical agents. In this current world colon cancer is the most common cancer when compare with other types of cancer. Throughout the world 1.2 million patients are detected for colon cancer in every year. In United States colon cancer is the second major causes for the death of both men and women. Cancers in the large and small intestine are the leading contributors for cancer morbidity and mortality in the world. In India the rate of colon cancer incidence was low in the past, but recently the incidence was increasing 35000 patients are suffering from colon cancer out of 3.5 million cases of cancer. Worldwide cancers are the second cause of death in the 21st century. Though several synthetic agents have been used for the treatment of cancer, due to their toxicity still research is going on for investigating chemotherapeutic agents of plant extract. In this Colon cancer occupies third position it is the most common cancer among the other. The presented study was determined to elucidate the in vivo anticolon cancer activity of *Coldenia procumbens*. Materials and Methods: The anticolon cancer effect of *Coldenia procumbens* against 1,2- dimethylhydrazine (DMH) induced coloncancer in male wistar albino rats. Results: DCP (Dichloromethane extract of *Coldenia procumbens*) which is a plant extract, at a dose of 200 and 400 mg/kg is found to decrease aberrant crypt foci (ACF) significantly ($P<0.001$). When compared to disease control (DMH), the weight of individual organs and hematological parameters have been increased significantly ($P<0.001$), which indicates the weight gain. Similarly, the total cholesterol level which is a biochemical parameter has been seen to be decreased significantly ($P<0.01$). We concluded that based on the above results Dichloromethane extract of *Coldenia procumbens* it has shows potent anti cancer activity.

Keywords :-DCP - Dichloromethane extract of *Coldenia procumbens*, ACF-Aberrant Crypt Foci DMH-1, 2-Dimethyl Hydrazine.

Access this article online	
Home page: http://www.mcmed.us/journal/abs DOI: http://dx.doi.org/10.21276/abs.2018.5.2.7	Quick Response code

Received:25.09.18	**Revised**:12.10.18	**Accepted**:15.07.18

INTRODUCTION

Colon cancer is one of the common most cancers in the world. Throughout the world 1.2 million patients are detected for colon cancer in every year. In United States colon cancer is the second major causes for the death of both men and women. Cancers in the large and small intestine are the leading contributors for cancer morbidity and mortality in the world. In India the rate of colon cancer incidence was low in the past, but recently the

Corresponding Author**K.Ramachandran** Email: - rcrama911@gmail.com

incidence was increasing 35000 patients are suffering from colon cancer out of 3.5 million cancer cases [1]. The colon or rectum is usually surrounded by epithelial cells, when these epithelial cells shows abnormal or proliferative growth, it results in Colorectal cancer. Most of this type of polyps or small growths of cells are benign, but, some may be potential to be cancerous in further consequences. It is estimated that the risk for colorectal cancer [2] is nearly two thirds by colorectal polyps which are pre-malignant. Higher success rates for treatment of cancer is prior screening and detection of cancer and increasing awareness and immediate removal of polyps before becoming cancerous. The main drawback in cancer patients is that by the time the patient is diagnosed with cancer, cancer would have been spread to other parts of the body due to lack of initial symptoms [3].

Every year Colorectal cancer is diagnosed worldwide around million of 1.2 people globally, it is the second most occurring cancer in women and the most third cancer in men. The cancer disease death is approximately 609,000 for each year (8% of all cancer deaths)[2], which made it as the fourth leading cause of death after stomach, liver and lung cancers. Approximately 430,000 new cases of colorectal cancers are identified every year in Europe making it the country with highest rate of colorectal cancer in the world. Following lung cancer, colorectal cancer is the second greatest cause of death in Europe, which accounts for about 12% of all types of cancers in Europe. In 2008, North America was made as second most cancerous region in the world with an incidence of about 177,000 new cases of colorectal cancers. Besides this, about 9% of cancer deaths and 11% of incidence of cancer has been accounted in North America.

Coldenia procumbens (Boraginaceae) [3] is commonly known as 'Creeping Coldenia'. It is a prostrate herb usually lying quite flat on the ground, common on dry rice grounds, stem reaching 10 -50 cm long, shaggy with white hairs, branches often numerous. In South India it is widely found distributed in trpical and subtropical zones. Leaves are crisped, alternate, and short and sessaile, rounded at the apex, warty hair with rosette of basal cells, veins 4-6 pairs on each side. In this study whole plant parts has been used.

Methods

Chemicals

DMH was purchased from Sigma Chemicals, Mumbai, India. After receiving, DMH was stored in a cool and dry place to prevent decomposition and contamination.

Preparation of Coldenia procumbens Extract

Fresh *Coldenia procumbens* were directly purchased from local region and District of Tiruvelveli, Tamilnadu, India in the month of April 2012. The botanical identity was confirmed and authenticated by a Taxonomist Dr. V.Chelladurai (Research Officer, Botany, (C.C.R.A.S) Government of India. The whole plant parts were then thoroughly washed, peeled, completely dried, coarsely, minced and made into a fine powder. The 150g of powdered plant material was packed in Soxhlet apparatus and refluxed with Dichloroethane until to get a clear solution, then the marc was pressed and this percolation of the plant extraction process was continued with other solvents Ethanol, Methanol and Water. The extracts of different solvents were concentrated to get dry the two extracts by evaporation.

Tumour induction

Just prior to use 1mM EDTA is added with DMH, and a value of 6.5 is adjusted for pH by added required quantity of 1mM sodium bicarbonate, pH is adjusted to ensure the chemical stability. At a dose of 20mg/kg body weight, in the groin of the animal, the subcutaneous injection of DMH has been given at a dosage of weekly once for a period of15 weeks [4].

Experimental animals

Male Wistar rats have been purchased from the National Institute of Nutrition, Hyderabad. Rats having age of 5 weeks and 150gm body weight were used for the study. All the rats were kept at room temperature of 22 ± 2^0 C under 12 hr dark-light cycles, humidity was maintained at60-70% in the animal house. Rats were fed with modified pellet diet, and water *ad libitum* freely throughout the study (including 1week for acclimatization). In accordance to the recommendations of CPCSEA all animal procedures were done with proper care and use of laboratory animals. The proposal of the present study was approved by IAEC of RVS College of Pharmaceutical Sciences, Coimbatore. (IAEC NO: IAEC/ 1012/ C/ 06/ CPCSEA approved on 24.12.2010)

Acute Toxicity Studies: Acute oral toxicity test wascarried out according to the OECD guideline No. 423. Before administration of drug Wistar Albino rats were subjected for overnight fasting. A dose of 2000 mg/Kg body weight of CG chloroform extract has been administered for three animals. Animals were kept under observation to note any changes in behavior, hypersensitivity relations etc. Over a period of 2 weeks, any mortality of animals was determined [5].

Experimental Design

After the administration of DMH, the animals were grouped into four groups of six animals in each. One group of animals were treated as control received normal saline only, out of three, two groups received 200 & 400mg/kg dose of DCP for 30 weeks [6].

During the course of study individual animal body weight was recorded, weekly. And % difference in the weights

between the groups was calculated.

Group 1	Normal saline PO daily up to 30 weeks
Group 2	DMH only (weekly once) up to 15 weeks
Group 3	DMH (weekly once) + DCP extract (200mg/kg dose), PO daily up to 30 weeks
Group 4	DMH (weekly once) + DCP extract (400mg/kg dose). PO daily up to 30 weeks

At the end of 30th week, all the experimental animals were left for overnight fasting and killed by cervical dislocation after termination of the study. Gross tumours were conducted by split opening the colon longitudinally. Appropriate buffer is taken in a tissue homogenizer and are weighed and homogenized, followed by immediate transfer of collected tiisue samples to the ice cold homogenized containers [7].

***In vivo* Methods**

The estimation of Protein was done by the procedure of Lowry *et al.* (1951).Colured complex formed when protein reacts with Folin-Ciocalteau reagent. The formation of colour complex was formed due to the chemical reaction of alkaline copper with protein and the reduction of phosphomolybdate by the tyrosine and tryptophan formation in the protein. The presence of aromatic amino acids depends on the intensity of color formation.

The estimation of Superoxide dismutase (SOD, EC.I.15.1.1) was done by using the procedure of Kakkar et al (1984) based on the 50% inhibition of the presence of NADH -phenazine methosulfate-nitroblue tetrazolium formazan at 520 nm. For inhibition of 50% of NBT reduction/min/mg protein about one unit of enzyme is added and assumed as the amount of enzyme required.

The estimation of catalase (CAT, EC.1.11.16) was done via the procedure of Sinha (1972). In the presence of hydrogen peroxide ($H_2 O_2$) and perchromic acid as an unstable CAT intermediate, chromic acid s prepared by reducing Dichromate in acetic acid by using heating method. By using 590 nm the formation of chromic acid was measured.

With a slight modification of Rotruck et al *(*1968) Glutathione peroxidase (GPx, EC.1.11.1.9) determination activity was done. In the presence of GSH and with H_2O_2 a known amount of enzyme preparation was incubated for a specific period of time. The required quantity of H_2O_2 for this is prepared by using Ellman (1959) method. The expressed values are as µmoles of GSH utilized/min/mg protein.

The determination of Reduced glutathione (GSH) content was measured via the procedure of Ellman (1959). When 5, 5' dithio (2-nitro benzoic acid) (DTNB) is added to sulfhydryl group containing compounds yellow color is developed, based o this principle, the GSH is determined. By using the units like mg/g tissue, the values are expressed.

By using the method of Devasagayam et al (2003), measurement of the levels of thiobarbituric acid reactive substances (TBARS) in tissues is estimated by lipid peroxidation was done. When thiobarbituric acid reacts with malondialdehyde, the pink chromogen was formed, 532 nm is used to estimate the secondary product of lipid peroxidation. By using the terms like nmoles/100g tissue, the values are expressed.

All the colons of the rats used in the experiment were collected by sacrificing them at the end of 30th week. To expose the luminal surface, all the colons are cut longitudinally and are flushed by using potassium phosphate buffer. The opened colons were placed between the filter papers and placed in 10% formalin fixative overnight, and then placed the 2cm long segments in a petridish and stained with 0.2% methylene blue solution. And total number of aberrant crypt per focus was counted.

Histopathology

The collected organ was washed with normal saline to remove the cell debris and preserved in 10% buffered neutral formalin solution, the tissues are trimmed to 2-3 mm thickness & subjected to preparation of paraffin blocks and cut in to 5µ thickness & followed H&E staining, the alterations in the tissue was read and reported

Statistics

Data collected from the above specified studies were subjected to One-way ANOVA followed by Dunnet's comparison by using Graph pad prism 5. Version 5.01.

The significance was expressed as ***P<0.001, **P<0.01, *P<0.05, #-Non Significant.

RESULTS

Acute Toxicity Study: Acute Oral Toxicity Study results shown in Table 1. In this DCP were given to the animal at different concentration of 5 mg/kg, 50 mg/kg, 300 mg/kg, 2000 mg/kg and the response like motor activity, pupils, urination, salivation, skin color etc., are all normal when compared with the control group animals. And this study revealed the non-toxic potential of the extracts were tested

Estimation of Tumor Markers results were shown in Table 2, and figure 1. In this DMH + DCP of two different concentrations of 200 mg/kg and 400 mg/kg were more significance than the DMH treated group. DMH + DCP 200 mg/kg 0.5000 ± 0.005733, DMH + DCP 400 mg/kg 0.4633 ± 0.01856.

Estimation of Carcinoembroyonic Antigen results of DCP results were shown in Table 3 and figure 2. In this DMH + DCP of two different concentrations of 200 mg/kg and 400 mg/kg were more significance than the DMH treated

group. DMH + DCP 200 mg/kg 0.2660 ± 0.006083, DMH + DCP 400 mg/kg 0.2177 ± 0.002404.

In-vivo anti-oxidant activity in colon tissue for DCP results were shown in Table 4 and figure 3.1 to 3.6. In this DCP extract showed significant anti Oxidant activity than the DMH treated group.

In-vivo anti-oxidant activity in liver tissue for DCP results were shown in Table 5 and figure 4.1 to 4.6. In this DCP extract showed significant anti-Oxidant activity than the DMH treated group.

Gross necropsy study revealed that DCP reduced the tumour formation in the dose dependent manner and 200 mg/kg & 400 mg/kg treated showed significant anticancer activity (plate 6,7,8).

Enumeration of ACF & Polyps for DCP results were shown in Table 6 and figure 5. In this DCP extract showed less significance compare to that of control group. Histopathological studies showed the significant reversal of the carcinogenesis in DCP treated groups with 200 mg/kg & 400 mg/kg treated groups when compared to control group, DMH alone treated group.

Table 1: Screening of Acute Oral Toxicity Study Result

S. No	Response	Concentration of DCP			
		5mg/kg	50mg/kg	300mg/kg	2000mg/kg
1	Motor activity	Normal	Normal	Normal	Normal
2	Grooming	Absent	Absent	Absent	Absent
3	Touch response	Absent	Absent	Absent	Absent
4	Torch response	Normal	Normal	Normal	Normal
5	Pain response	Normal	Normal	Normal	Normal
6	Tremors	Absent	Absent	Absent	Absent
7	Convulsion	Absent	Absent	Absent	Absent
8	Righting reflux	Normal	Normal	Normal	Normal
9	Gripping strength	Normal	Normal	Normal	Normal
10	Pinna reflux	Present	Present	Present	Present
11	Corneal reflux	Present	Present	Present	present
12	Writhing	Absent	Absent	Absent	Absent
13	Pupils	Normal	Normal	Normal	Normal
14	Urination	Normal	Normal	Normal	Normal
15	Salivation	Normal	Normal	Normal	Normal
16	Skin colour	Normal	Normal	Normal	Normal
17	Lacrimation	Normal	Normal	Normal	Normal

Estimation of tumor markers

Table 2: Estimation of Alpha-Feto-Protein (AFP) for DCP

GROUPS (n=6)	CONTORL	Only DMH	DMH+DCP (200 mg/kg)	DMH+CP (400 mg/kg)
AFP (ng/dL)	0.4800 ±0.01528	0.6780 ±0.007234	0.5000 ±0.005773***	0.4633 ±0.01856***

***P<0.001, **P<0.01, *P<0.05, #-Non Significant

Data is expressed as Mean±SEM. (n = 6, animals in each group).

Statistical comparison: One way ANOVA, followed by Dunnet's comparison was performed.

DMH only (Group 2) was compared with Normal control (Group 1), DMH+MCP(200 mg/kg) Group 3, DMH+MCP(400 mg/kg) Group 4, the values of Alpha-Feto-Protein was expressed as ng/dl.

Table 3: Estimation of Carcinoembroyonic Antigen (CEA) for DCP

GROUPS (n=6)	CONTORL	Only DMH	DMH+DCP (200 mg/kg)	DMH+DCP (400 mg/kg)
Carcinoembroyonic Antigen (ng/dL)	0.1953 ±0.002906	0.4733 ±0.007126	0.2660 ±0.006083***	0.2177 ±0.002404***

***P<0.001, **P<0.01, *P<0.05, #-Non Significant

Data is expressed as Mean±SEM. (n = 6, animals in each group).

Statistical comparison: One way ANOVA, followed by Dunnet's comparison was performed.

DMH only (Group 2) was compared with Normal control (Group 1), DMH+MCP(200 mg/kg) Group 3, DMH+MCP(400 mg/kg) Group 4, the values of Carcinoembroyonic Antigen was expressed as ng/dl.

Table 4: In-Vivo Anti-Oxidant Activity in Colon Tissue for DCP

GROUPS (n=6)	CONTORL Group-I	Only DMH Group-II	DMH+DCP (200 mg/kg) Group-III	DMH+DCP (400 mg/kg) Group-IV
PROTEIN Mg/G Tissue	0.4900 ±0.02309	0.2390 ±0.02610	0.4040 ±0.007024**	0.4117 ±0.01922***
SOD (units/min/mg protein)	5.460 ±0.2272	2.827 ±0.08090	3.380 ±0.02646***	3.930 ±0.09849***
CATALASE (moles of H2O2 consumed/min/mg protein)	41.60 ±0.863	28.46 ±0.7681	24.40 ±0.2797**	28.41 ±0.7664#
GPx Gram/mg protein	257.8 ±6.693	84.40 ±1.080	259.7 ±3.869***	233.5 ±14.17***
GSH Glutathione Gram/mg protein	107.6 ±5.205	79.97 ±4.383	97.11 ±10.26**	106.6 ±12.89**
LPx MDA formed/mg protein	103.2 ±3.215	54.89 ±2.422	92.14 ±1.138***	101.2 ±1.273***

***P<0.001, **P<0.01, *P<0.05, #-Non Significant. Data is expressed as Mean±SEM. (n = 6, animals in each group).
Statistical comparison: One way ANOVA, followed by Dunnet's comparison was performed. DMH only (Group 2) was compared with Normal control (Group 1), DMH+DCP (200 mg/kg) Group 3, DMH+DCP (400 mg/kg) Group 4.

Table 5: In-Vivo Anti Oxidant Activity in Liver Tissue for DCP

GROUPS (n=6)	CONTORL Group-I	Only DMH Group-II	DMH+DCP (200 mg/kg) Group-III	DMH+DCP (400 mg/kg) Group-IV
PROTEIN Mg/G Tissue	06433 ±0.02906	0.2700 ±0.02646	0.5610 ±0.01950***	0.5633 ±0.02186***
SOD (units/min/mg protein)	7.717 ±0.1989	2.437 ±0.0581	4.043 ±0.06642**	4.183 ±0.1126***
CATALASE (moles of H2O2 consumed/min/mg protein)	51.01 ±1.324	24.15 ±0.5355	32.67 ±0.2805**	37.17 ±0.2443**
GPx Gram/mg protein	207.6 ±5.485	73.74 ±2.681	189.9 ±12.42***	174.3 ±8.486***
GSH Glutathione Gram/mg protein	130.1 ±9.655	81.27 ±4.816	99.53 ±1.048#	118.8 ±3.627**
LPx MDA formed/mg protein	92.15 ±1.791	68.14 ±2.752	85.17 ±1.530#	90.14 ±1.609***

***P<0.001, **P<0.01, *P<0.05, #-Non Significant. Data is expressed as Mean±SEM. (n = 6, animals in each group).
Statistical comparison: One way ANOVA, followed by Dunnet's comparison was performed.DMH only (Group 2) was compared with Normal control (Group 1), DMH+MCP (200 mg/kg) Group 3, DMH+MCP (400 mg/kg) Group 4.

Table 6: Enumeration of ACF & Polyps for DCP

GROUPS (n=6)	CONTORL	Only DMH	DMH+DCP (200 mg/kg)	DMH+DCP (400 mg/kg)
ACF & Polyps	0.0 ±0.0	13.33 ±1.856	6.667 ±1.202**	5.000 ±0.5774**

***P<0.001, **P<0.01, *P<0.05, #-Non Significant. Data is expressed as Mean±SEM. (n = 6, animals in each group).
Statistical comparison: One way ANOVA, followed by Dunnet's comparison was performed.
DMH only (Group 2) was compared with Normal control (Group 1), DMH+DCP (200 mg/kg) Group 3, DMH+DCP (400 mg/kg) Group 4.

Fig.1 Estimation of Alpha-Feto-Protein

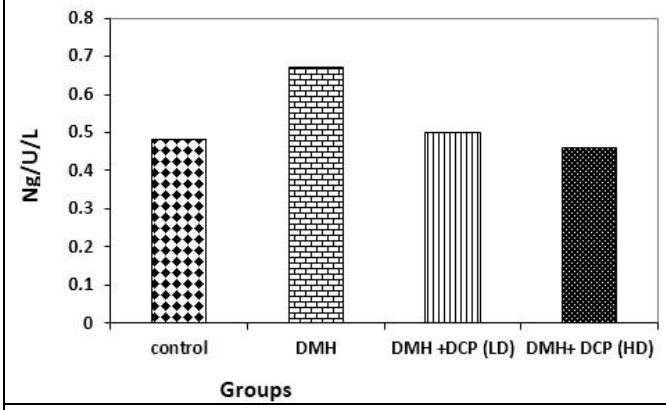


Fig.2 Estimation of Carcinoembroyonic Antigen (CEA)

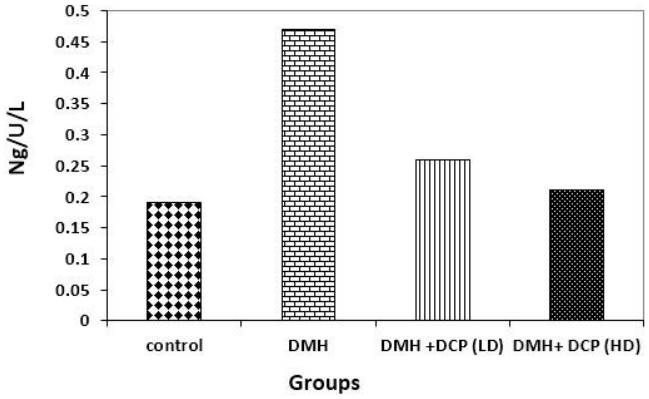


Fig.3.1 Estimation of Protein (Colon)

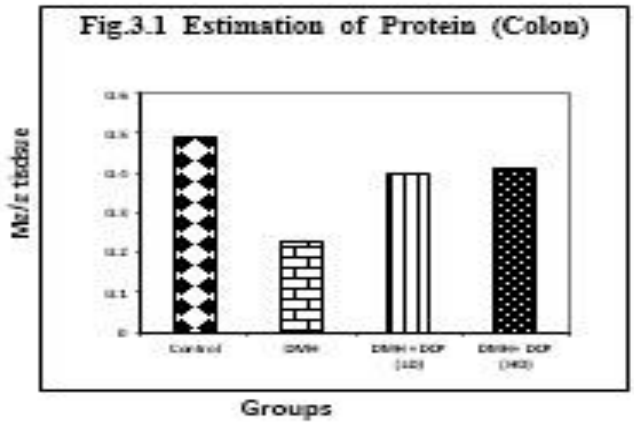


Fig.3.2 Estimation of SOD(Colon)

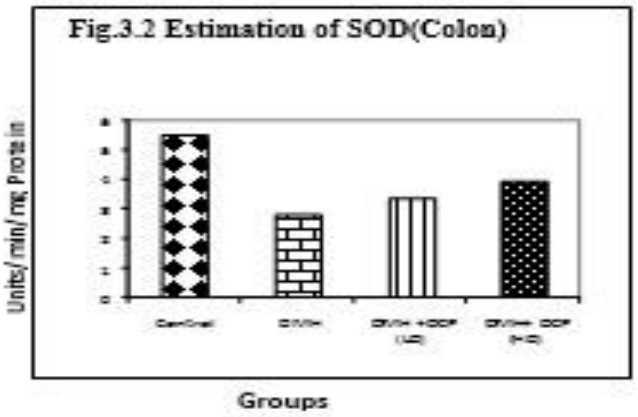


Fig.3.3 Estimation of Catalase (Colon)

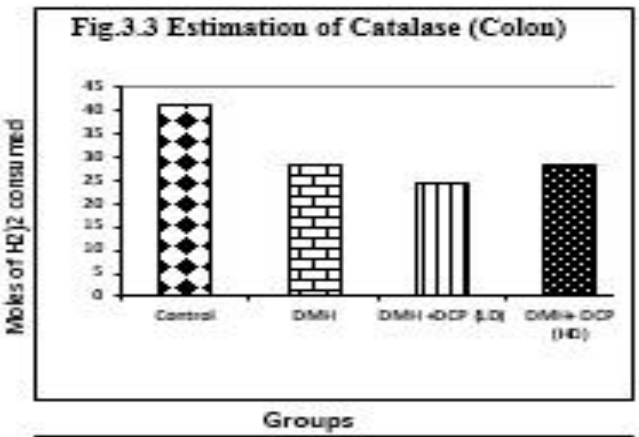


Fig.3.4 Estimation of GPX (Colon)

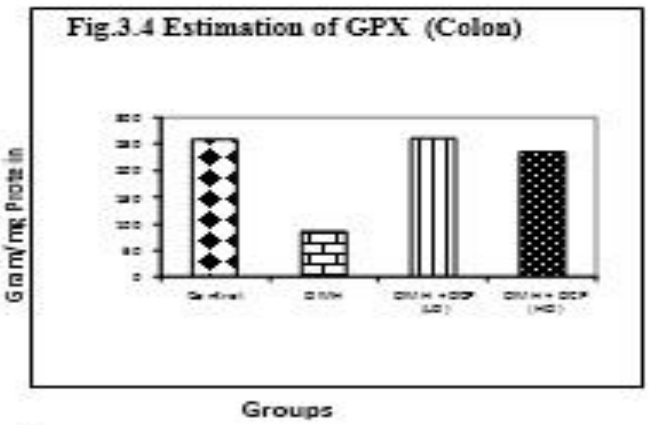


Fig.3.5 Estimation of GSH (Colon)

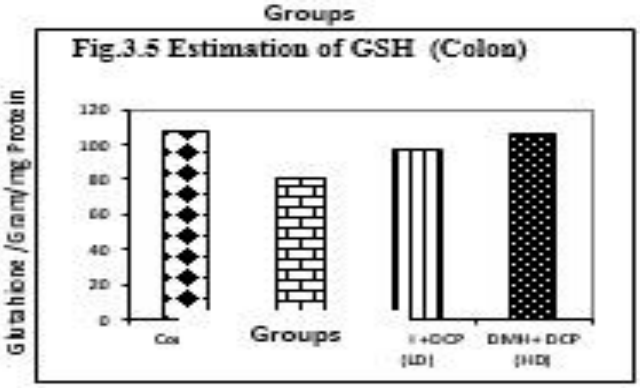
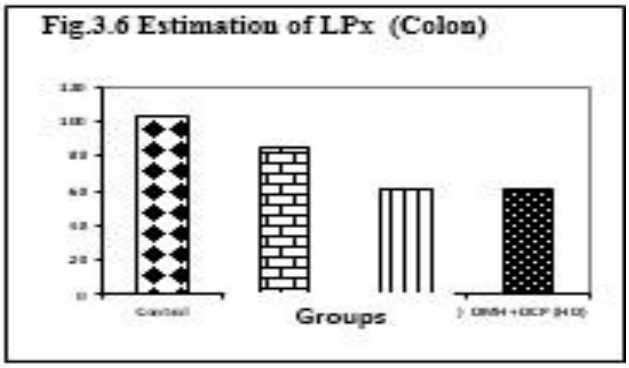
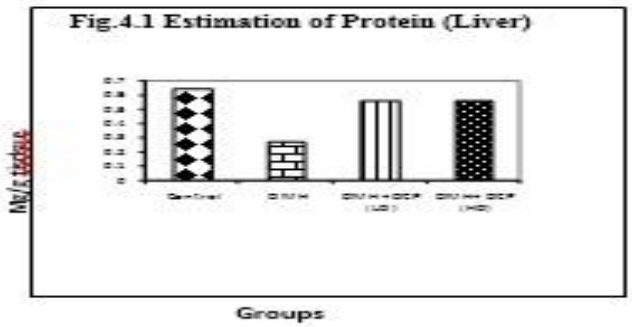
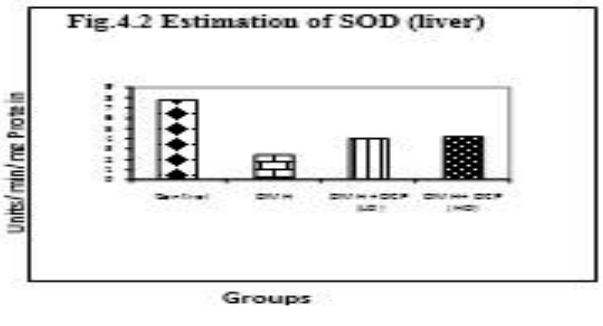
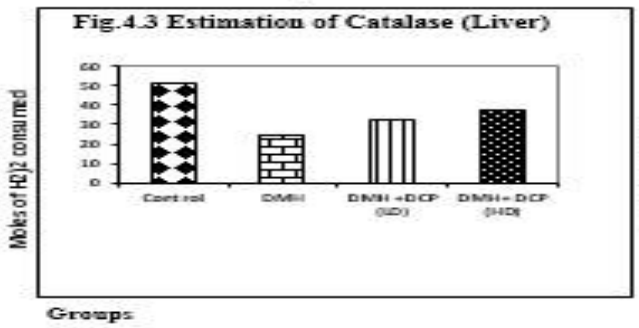


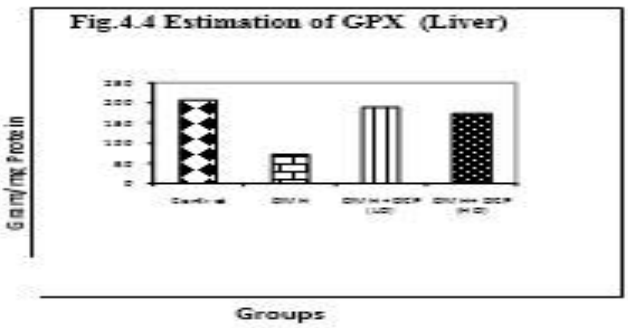
Fig.3.6 Estimation of LPx (Colon)

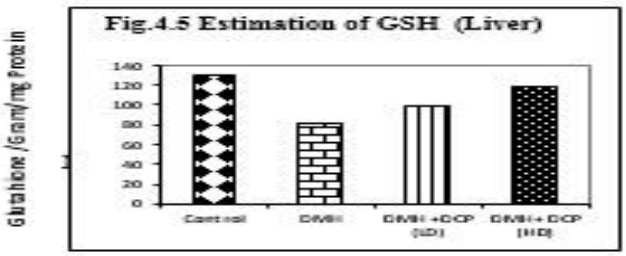


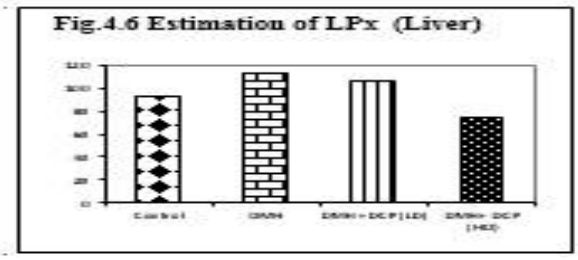












5. Gross pathological changes. Gross Pathological changes occurred in colon tissue of DMH treated groups (aberrant crypt foci).

Plate No. 6. DMH only treated group

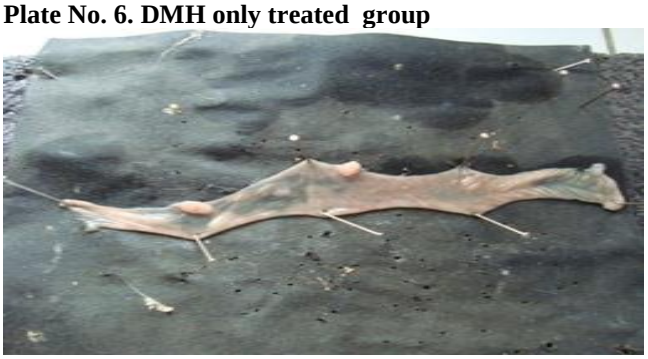


Plate No 7. DCP (200 mg/kg) treated

Plate No 8. DCP 400 mg/kg) treated group

Fig.7. Enumeration of ACF & Polyps

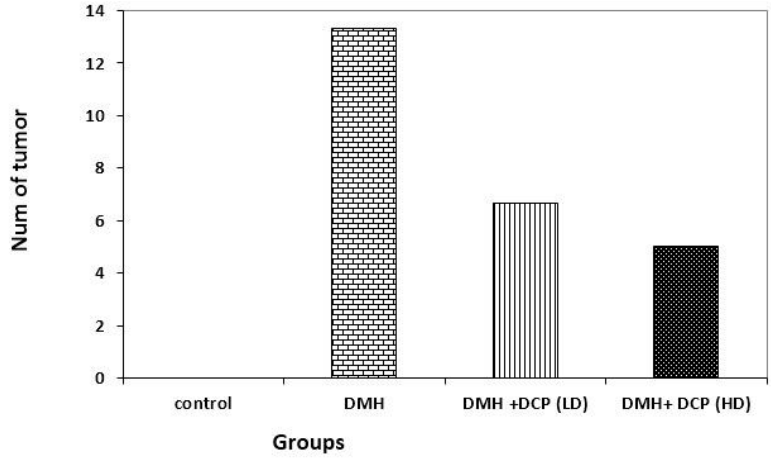


Plate No 9. Colon show no obvious abnormality in control group

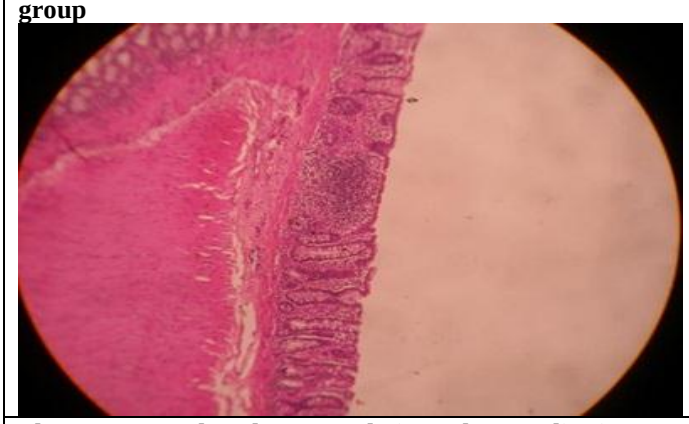


Plate No. 10. DMH treated colon tissue shows the presence of tiny pednucleated polyp probably a benign tubular adenoma

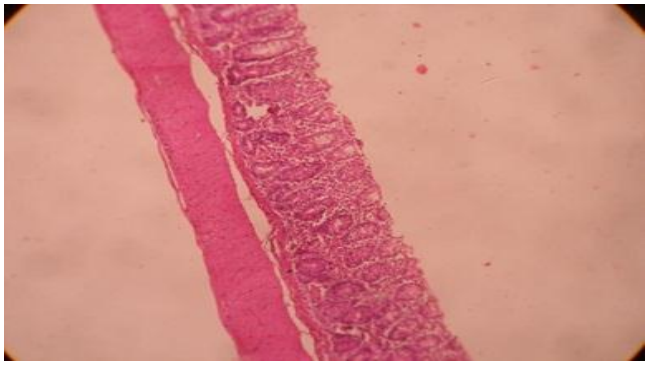


Plate No. 11. colon shows no obvious abnormality in DCP (200 mg/kg) treated

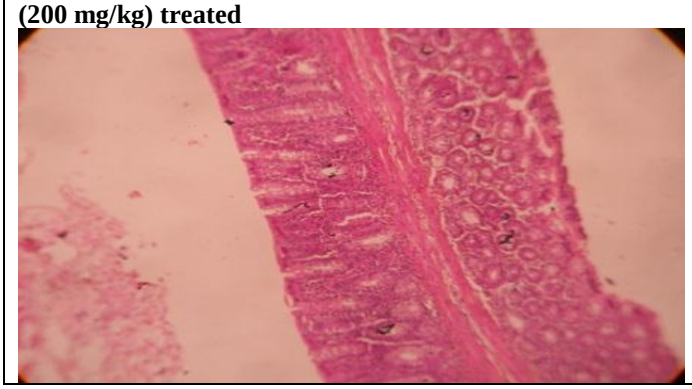
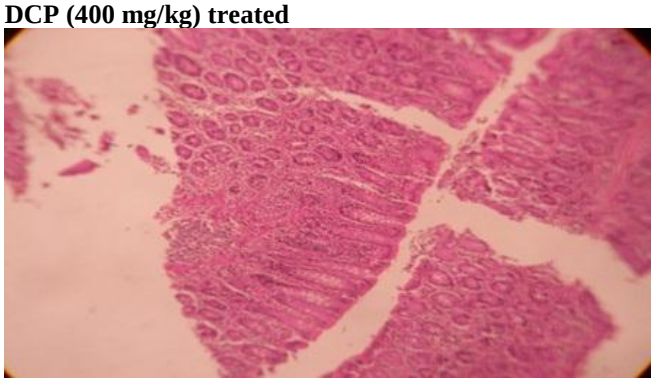


Plate No. 12. colon shows no obvious abnormality in DCP (400 mg/kg) treated



DISCUSSION

There are many types of malignancies in the world; in this the most common one is colon cancer which has seen in many regions of the world. Also it is the major cause of death in both men and women as well. The major causes of this is due to a pathological consequence of persistent oxidative stress, leading to DNA damage, mutations in cancer related genes, as well as epigenetic silencing of tumor suppressor genes the consequence of such a genomic instability is cellular overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS-induced carcinogenesis participate may be due to the damage of Oxidative DNA. Lipid peroxidation is a paradox of aerobic life, affecting man's health and the quality of modern life. The auto oxidation protection by endogenous enzymatic or nonenzymatic mechanisms in the biological systems was susceptible to lipid rich matrices. The effect of Dichloromethane, which is ad extract of *Coldenia procumbens Linn* on the cancers induced by 1, 2-dimethyl hydrazine, was studied in vivo in

the present study, which was the major effort and aim of the study.

In the present model of 1, 2-dimethyl hydrazine induced colon cancer, the anti- cancer effect of DMH was assessed by using a dose of 20mg/Kg of DMH over a period of 15 weeks. And the efficacy of the extract was evaluated by treating the animals with two dose levels one week prior to DMH treatment and simultaneous treatment with DMH for 30 weeks by daily dosing.

At the end of 31 weeks treatment, hematological, biochemical parameters and plasma tumor markers were estimated.

In the cancer condition there will be a significant change in serum biochemical parameters. There will be a significant decrease in triglycerides and total cholesterol and significant increase in SGOT, SGPT, bilirubin levels.

Many Physicians and public health workers have been intrigued in recent years with the possibility that modification of the diet may result in a reduced incidence and mortality from Cancer. Renewed attention is made on the possible role of dietary and endogenous lipids in understanding the etiology and prognosis of cancer by the recent reports. In the etiology of coronary heart disease, cholesterol which is recognized as the major factor , in the recent study period of cancer, it has also become the major factor for cancer etiology.

Due to the presence of intrinsic limitations and a solely retrospective focus [7] it has been criticized for the epidemiologic data in the role of cholesterol involvement in cancer. In a recent study, a positive association was noted between serum cholesterol levels and the risk for rectal cancer in men [8,9].

From the recently published articles on the epidemiology f cancer, the increased risk of death from cancer has shown a data with patients revealed with presence of low plasma cholesterol levels. Although several authors proposed that hypo cholesteremia is a predisposing factor for cancer development, no causative relation has been established so far. [10,11]

However, some authors believe that hypocholesteremia is in fact the result rather than the cause of cancer [12,13].

The association of various factors like levels of serum cholesterol and triglycerides in dietary factors or basic constitutional factors related cancers have been of great interest in finding the etiology of cancer by the current theories regarding cancer causation. Curiously enough very few studies exist concerning serum lipid profile in patients with cancer [14,15].

The present study examined the lipid profile of animals with colon cancer in comparison with DCP treated cancer groups there was a significant increase in the total cholesterol level due to modification in the diet. The effects of DCP on different serum biological parameters (Table 5; Figure 4.1-4.6) are showing significant decrease in serum biological parameters.

This protein may appear n the blood of some people presenting with certain cancers like cancers in large intestine like colon or rectal cancers can be tested by using carcinoembryonic antigen (CEA) test. It may also be present in people with cancer of the pancreas, breast, ovary, or lung.

During the development of fetus the CEA is normally produced, and it is not usually present in the healthy adults as it stops the production of CEA before birth. Alpha-feto-protein is a serum protein that is detected in elevated concentration in carcinoma conditions; it is a serum protein similar in size, structure to serum albumin. The levels of AFP will be in minute quantities in adults where there will be an elevated level in cancer condition [16].

In the present study, a decrease in the level of CEA and AFP was observed followed by DCP treatment(Table 20, 21; Figure 18, 19) indicates a positive prognosis the decrease levels on DCP treatment prevents the neoplastic growth and reduces the level of carcinoma, which indicates that it possesses anticarcinogenic properties [17].

In the present study there was significant change in the protein levels due to impairment of glyconeolytic enzymes when compared with treated and controlled group. Groups treated with DCP shows a significant increase in the altered protein levels (Table 5; Figure 4.1-4.6).

Alterations or modification in the oxidation and antioxidant profile are known to happen in the cancer tissues. The reports of the current study clearly elucidates that the administration of the pro-carcinogen DMH with the presence of DCP brings about profound alterations or modifications in the tissue lipid peroxidation and antioxidant status. Oxidative stress is due to the formation of damage happened by free radical attack on cellular macromolecules such as lipids and DNA. Through several processes, like elicit oxidative stress, an active electrophilic carbonium ion is produced in the liver by DMH by undergoing activation of metabolism in the liver, in turn DMH is a carcinogen. Recent evidence has indicated that the generation of reactive oxygen species may be involved in various carcinogenic processes [18].

The levels of lipid peroxidation were measured in the liver, colonic tissues of DMH treated rats were significantly higher in the liver when compared with the control, whereas the levels of these lipid peroxidation products were significantly lowered in the DMH alone treated group (Table 18, 19; Figure 16, 17). Cancer cells acquire particular proliferative characteristics when the level of lipid peroxidation is low in cancer cells, which was observed in many rats presented with cancer, also various studies have shown reduced levels of lipid peroxidation in tumour tissue of rats in various types of cells [19]. Thereby, increased proliferation was observed in rats treated with DMH, which indicates colonic lipid

peroxidation. Therefore, this is the reason for malignant tissues being less susceptible and more resistant for free radical attacks. It also may be due to decreased susceptibility and/or increased resistance of target organs for free radicals resulting in the decreased levels of lipid peroxidation in DMH treated animals.

Previous studies in our lab have shown similar results. The lipid peroxidation levels of diseased rats have neared to that of control rats when DCP is administered to the DMH treated rats, which was observed in the present study [20], which may be due to the potent scavenging free radical properties exhibited by DCP Phenolic hydroxyl group by its strong oxidation properties. Thus the phenolic hydroxyl groups present in DCP may be responsible for its antioxidant properties. Moreover the role of DCP in protecting cells from the DMH induced loss of their oxidative capacity, may be attributed to the anti proliferative activity of DCP.

Superoxide and hydroxyl ions are the two major toxic oxygen free radicals, which are now suppressed by two important enzymic antioxidants namely SOD and CAT. CAT prevents oxidative damages by catalyzing the formation of H2O and O2 from H2O2. A previous study by Slaga*et.al,* (1995) has shown that carcinogen administration usually minimizes the activities of SOD and CAT. Earlier laboratory reports have also shown similar results [21,22]. In our present study we observed a similar trend in theSOD and CAT activities of the liver, colonic tissues on DMH administration. DCP administration to DMH treated rats significantly normalized the SOD and CAT activities. This may be due to the free radical scavenging and antioxidant property of DCP which is supposed to be achieved by its trapping O2 to form stable radicals of it.

In conjugation with GPx, GSH is one of the important thiol, which scavenges reactive oxygen species by protecting the cells from cytotoxic and carcinogenic chemicals. This being antripeptide also helps in detoxifying many environmental carcinogens and free radicals. Four selenium cofactors are present in the GPx enzyme that catalyzes the breakdown of organic hydroperoxides and H_2O_2. By the process of oxidation of NADH to NAD+ is brought by conversion of oxidized glutathione (GSSG) by a glutathione regenerating enzyme namely GR. In consistent to previous results (Vennila*et.al,* 2009), the GSH, GPx levels of the liver, colonic tissues have been observed to be decreased in DMH alone treated rats in comparison of control rats in the present study. May be due to the involvement of GSH, GPx on DMH administered group, the decline in the levels of GSH is seen by the mechanism of detoxification and possible repair mechanism in the colonic mucosa and intestinal mucosal cell lines. Increased detoxification capacity may be influencing the decrease in GPx enzyme activity due to some adaptive mechanism by which selective growth advantage is gained by the tumor cells over the surrounding normal cells as an advantage. The levels of GSH and the level of activity f GPx have been increased in DMH treated rats by administration of DCP (Table 18, 19; Figure 16, 17). In determining the potency of many anti carcinogenic substances have been evaluated by the inductions produced by these enzymes. DCP is known to suppress reactive oxygen species and enhance the levels of GSH. Thus the elevated levels/activities of GSH, GPx in DMH treated rats on DCP supplementation may be due to the combined effects of the phenols which can help in scavenging free radicals

Aberrant crypt foci (ACF) are early morphologic changes observed in rodents after administration of colon-specific carcinogen such as azoxymethane. With sporadic and inherited forms of colon cancer, at high frequency the similar lesions were observed in the colons of the patients. ACF are currently used as surrogate biomarkers which are also considered as pre-neoplastic lesions. These surrogate biomarkers helps in rapidly evaluating both naturally and synthetically occurring chemo preventives. It was clearly studied by using AOM in the Fisher 344 rat models. In this it accurately replicates many of the clinical, genetic, cellular, and morphologic features of human colorectal cancer. Increase in the size of pericryptal zone, epithelial cell linings, size of crypts are the characteristics of AOM-induced ACF, which share many biochemical and morphological characteristics with tumours, which includes comparable increase in cell proliferation [24,25].

In several human colon cancer cell lines and chemically induced rodent cancers the PPARγ are more aberrantly expressed. It can be up-regulated by treatment with butyrate, which induces differentiation of colon cancer cell line, Caco-2 cells. Mutations in PPARγ gen is often associated with development of colon cancers. In addition, it is reported that NSAIDs could bind and activate PPARγ , reported by Lehmann *et.al,* and provided a preventive effort for reduction of use of these drugs in patients with colon carcinogenesis. PPARα are also found to be activated by NSAIDs [26,27].

The effects of extracts on DMH induced colon cancer was evaluated by the formation of aberrant crypt foci (ACF). After the termination of the study the no of ACF in the colon was enumerated to determine the effect of extracts on DMH induced colon cancer. From the present study the extract treated groups shown significant reduce in the formation of ACF. (Table 24; Figure 28)

Histopathology reports showed a DMH treated colon tissue shows the presence of tiny pednucleated polyp probably a benign tubular adenoma, and the DCP treated groups does not showed any abnormality And so it suggested that DCP have shown a good response when compared with the first group.

The restoration of normal hematological levels, *in vivo* anti oxidant system, glycolytic enzymes, and the results obtained from the *in vitro* anti oxidant studies as well as the effects on colon cancer and body weight and

also the histopathology reports are the suggestive of the potential chemo preventive activity of DCP against the DMH induced colon cancer and need a further studies for the potential development of more active compounds as an effective chemo preventive agent against the colon cancer[27-29].

CONCLUSION

With the above said findings we conclude that the plant *Coldenia procumbens Linn* possess anti-oxidant and anti-colorectal cancer activity, In this *Coldenia procumbens Linn* possess significant anti-cancer activity compare to that of DMH treated group, thorough toxicological profile has to be determined on the crude extracts as well as on isolated compounds to confirm the safety of the drug.

ACKNOWLEDGEMENT

The authors are grateful to DR R Venkatnarayanan Principal, RVS College of pharmaceutical Sciences, Sulur, Coimbatore and my Guide Dr EP Kumar from Karpagam College of Pharmacy for providing the necessary facilities to carry out the study.

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Cite this article:
Ramachandran K, Venketnarayanan R. Evaluation of Anticancer Activity of *Coldenia procumbens* Linn In 1, 2-Dimethyl Hydrazine Induced Colon Cancer On Rat Model. *Acta Biomedica Scientia*, 2018;5(2):71-82. DOI: http://dx.doi.org/10.21276/abs.5.2.6