



CODEN (USA): IAJPBB

ISSN: 2349-7750

**INDO AMERICAN JOURNAL OF  
PHARMACEUTICAL SCIENCES**Available online at: <http://www.iajps.com>

Research Article

**PHARMACOLOGICAL EVALUATION OF  
ETHANOLIC EXTRACT OF WHOLE PLANT OF CASSIA  
ABSUS ON 1,2 DIMETHYL HYDRAZINE INDUCED COLON  
CANCER IN MICE.**Athiya Sulthana\*<sup>1</sup>, A. Ravi kiran<sup>1</sup>, Suneetha Gowdu.R<sup>2</sup>, K. Vanitha Prakash<sup>2</sup><sup>1</sup> G. Pulla Reddy College of Pharmacy, Hyderabad-28, Telangana., India.<sup>2</sup> SSSJ College of Pharmacy N Pally, Hyderabad-500085.**Abstract:**

**Background:** Colon cancer is the most common cancer and fourth most frequent cause of cancer deaths worldwide. 1,2 dimethyl hydrazine (DMH) is a toxic environmental pollutant which as reported as a specific colon procarcinogen. Aberrant Crypt Foci (ACF) in the colonic mucosa have been hypothesized to represent precursor lesions of chemically induced colon cancer. Cassia absus has been traditionally claimed to be an anti tumor agent but no studies has been done so far. Based on the traditional claims, we presumed that Cassia absus as a potential anti-oxidant and anti-tumor agent, may show ameliorating effect on DMH induced colon cancer in mice. **Objective:** The objective of the present study was to evaluate the ameliorating effect of ethanolic extract of whole plant of Cassia absus on DMH induced colon cancer in mice. **Method:** 30 mice (either sex) of 10 weeks old were divided into five groups (n=6) Group I - served as normal control group received vehicle p.o. Group II- disease control group received DMH (20mg/kg s.c.) twice a week for 2 weeks. Group-III and IV – drug treated group received DMH (20 mg/kg sc.) twice a week for 2 weeks and Cassia absus (200 and 400 mg/kg, p.o.) for 7 weeks. Group V received DMH (20 mg/kg s.c.) twice a week for 2 weeks and 5-fluorouracil (20 mg/kg i.p.) once daily up to 2 weeks. During the treatment period body weights was measured at weekly intervals. At the end of seventh week all the animals were sacrificed and their colons were isolated for quantification of Aberrant Crypt Foci, Biochemical estimations (MDA, GSH, Catalase, SOD) and Histological studies. **Results:** Induction of colon cancer by DMH administration significantly decreased high incidence of ACF, aberrant crypts and crypt multiplicity along with decreased anti oxidant levels and increased colonic MDA levels. Administration of Cassia absus resulted in the decrease in the number of ACF and crypt multiplicity, antioxidant enzymes levels which reflect a favorable balance between potentially harmful oxidants and natural anti oxidants which may be attributed to the chemo preventive effect of Cassia absus. **Conclusion:** In conclusion, the present study suggests that treatment with ethanolic extract of whole plant of Cassia absus exerts beneficial effect in DMH induced colon cancer in mice probably due to their anti oxidant and anti tumor properties.

**Key words:** 1,2 dimethyl hydrazine (DMH), Aberrant Crypt Foci, Cassia absus

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Please cite this article in press Athiya Sulthana et al , *Pharmacological Evaluation of  
Ethanolic extract of whole plant of cassia absus on 1,2 dimethyl hydrazine Induced Colon Cancer In Mice.,  
Indo Am. J. Pharm. Sci.*, 2015;2(8).

**INTRODUCTION:**

Colon cancer is the most common cancer and fourth most frequent cause of cancer death worldwide. It is one of the fastest emerging gastro intestinal cancers in the Asia Pacific region. It is estimated that 1 in every 20 people will develop colon cancer. The estimated colon cancer cases will be 96,830 till December 2014. There are about 600,000 worldwide deaths till 2014 due to colon cancer. In India there are about 3.5 million cases of cancer of which about 35,000 are found to suffer from colorectal cancer. Though the estimated incidence of colorectal cancer is low in India its prevalence is increasing at an alarming rate with changing life style. Incidence is around 53 per 1, 00,000 men and 51 per 1, 00,000 women worldwide. For 2012, estimated new cases of colon cancer are 103,170. [1-2]

Colon cancer begins as a benign polyp, a growth of tissue that starts in the lining and grows into the centre of the colon. These polyps are harmless growth on the inside of the intestines. They form when they are problems with the way the cells grow and repair the lining of the colon. The two most common types of polyps are adenomatous polyps and hyper plastic polyps. The benign polyp can develop into an adenoma with high-grade dysplasia eventually progressing to an invasive cancer. Adenomatous polyps develop into cancer over time, whereas hyper plastic polyps do not develop into colon cancer. Adenoma is considered to be a precursor lesion of colon cancer occurring rarely in individuals under 49 but becoming more prevalent later in life. Age, genetics, dietary components, smoking and alcohol consumption, personal history of colon polyps, family history of cancer and lack of exercise are the major risk factors associated with colon cancer<sup>3,4</sup>. Ethnic and geographical studies suggest that environmental factors may play an important role in colorectal cancer with rates higher in North American and northern Europe, lower in southern Europe, and much lower in Asia and Africa. The main symptoms of colon cancer include change in bowel habits, diarrhea, constipation, or feeling that the bowel does not empty completely, bright red or very dark blood in the stool, discomfort in the abdomen including frequent gas pains, bloating, fullness and cramps, weight loss with no known explanation, constant tiredness or fatigue, unexplained iron-deficiency anaemia [2]

**Stages of Colon Cancer:** The stage describes the extent of the cancer in the body. It is based on how far the cancer has grown into the wall of intestine, whether or not it has reached nearby structures, and whether or not it has spread to the lymph nodes or distant organs. The stage of a cancer is one of the most important factors in determining prognosis and treatment options. Stages should preferably be defined by TNM classification [3].

**AJCC or TNM Classification:**

The most commonly used staging system for colorectal cancer is that of the American Joint Committee on cancer (AJCC), sometimes also known as the TNM system. The TNM system describes 3 key pieces of information:

T describes how far the main (primary) tumor has grown into the wall of the intestine and whether it has grown into nearby areas.

N describes the extent of spread to nearby (regional) lymph nodes

M indicates whether the cancer has spread (metastasized) to other organs of the body.

(Colorectal cancer can spread almost anywhere in the body, but the most common sites are the liver and lungs.)

Numbers or letters appear after T, N and M to provide more details about each of these factors. The numbers 0 through 4 indicate increasing severity. The letter X means "cannot be assessed because the information is not available [ 4-5]

The most common treatment for colon cancer includes surgery (colectomy, polypectomy), radiation therapy and chemotherapy. Various chemotherapeutic drugs are used to treat colon cancer like 5-flourouracil, Leucovorin, Irinotecan, Bevacizumab and Cetuximumab depending on the stage of the disease but there is no specific therapy till date. As a result of the limitations of conventional therapy like life-threatening diarrhea, neuropathy, Immuno suppression. etc., there is a need to develop novel and safe therapeutic agents to treat colon cancer [6].

Colon carcinogenesis is known to be a pathological consequence of persistent oxidative stress, resulting in DNA damage and mutations in cancer associated genes in which the cellular over production of reactive oxygen species (ROS) have been Implicated. There is ample evidence that oxidative stress is increased in circulation and is closely related to the pathogenesis of oxidative damage mediated tumor genesis. It is well known that DNA damage is the initial step in carcinogenesis and also well established that many naturally occurring antioxidant compounds offers protection in preventing DNA damage and have cancer-preventive properties [7].

Previous studies suggested that a drug with anti oxidant and anti-tumor activity can be effective against colon cancer.

Ethnolic extract of *Cassia absus*, belonging to the family Fabaceae possess significant antioxidant and anti tumor properties. However, till date no studies have been reported on the ameliorating effect of Ethnolic extract of *Cassia absus* on DMH induced colon cancer in mice.[8-9-10].

**MATERIALS AND METHODS:****Experimental Animals**

Male Swiss albino mice (20-30g) were purchased from national institute of nutrition (NIN), Hyderabad. They were placed individually in clean, transparent polypropylene cages with free access to food and water with 12: 12 hr dark/light cycle is followed. They were acclimatized for a period of one week and divided into experimental groups.

All the experimental procedures were carried in accordance with the committee for the purpose of control and supervision of experiments (320/CPCSCEA dated 03-01-2001) on animals. The study was approved by the Institutional Animal Ethical committee (GPRCP/IAEC/11/13/3/PCL/AE-2-Mice-M/F-30)

#### Chemicals:

- 1,2-dimethyl hydrazine (procured from sigma Aldrich)
- (2-nitrobenzoic acid) (DTNB) were obtained from Sigma Aldrich.
- Tri chloroacetic acid (TCA) and Ellman's reagent were obtained from High media.
- Butanol, pyridine, Hydrogen peroxide, EDTA, epinephrine, and other chemicals were obtained locally.

#### Study Design:

##### DMH Induced Colon Cancer In Mice:

Swiss albino mice of either sex weighing about 20-30g were used. Colon cancer was induced by injecting DMH. DMH was dissolved in saline and

administered at a dose of 20 mg/kg i.p. twice a week for 2 weeks.

#### Experimental Design:

- Swiss albino mice of either sex were acclimatized to laboratory conditions are divided into 5 groups. (n=6).
- Group 1 (Normal control):** received saline for 7 weeks.
- Group 2 (Disease control):** received DMH (20mg/kg, s.c.) twice a week for 2 weeks
- Group 3 (Disease treated):** received cassia absus (200mg/kg) orally once daily for 7 weeks
- Group 4 (Disease treated):** received cassia absus (400mg/kg) orally once daily for 7 weeks
- Group 5 (standard treatment):** received DMH (20 mg/kg) twice a week for 2 weeks and 5-FU (20 mg/kg) i.p. once daily for 2 weeks.
- At the end of 7 week, all the animals were sacrificed by cervical dislocation and the colon were isolated for the quantification of Aberrant Crypt Foci, Biochemical estimations (MDA, GSH, Catalase, SOD) and Histological examination.

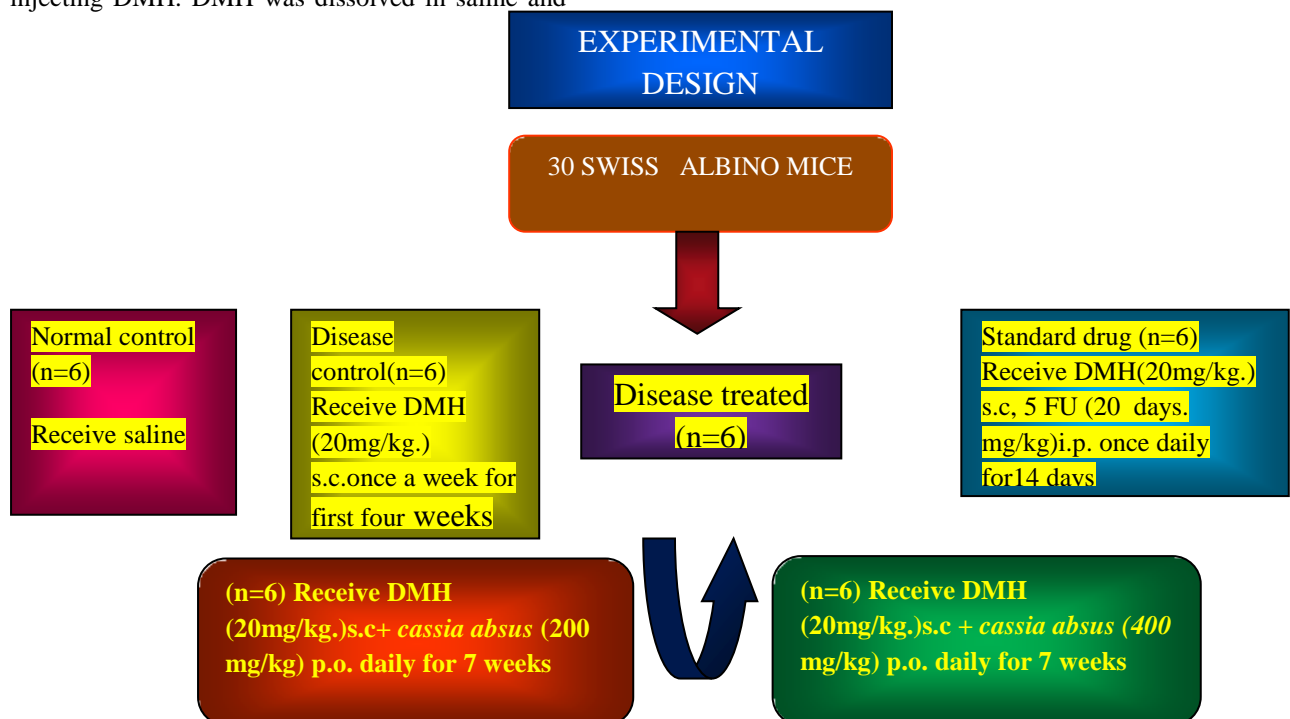


Fig 1: Experimental Design

**Evaluation of Parameters:****Determination of Aberrant Crypt Foci:**

Aberrant Crypt Foci (ACF) was counted by the method of bird. Isolated colons were flushed with phosphate buffered saline and opened along the longitudinal median axis, and fixed flat between two piece of filter paper in 10% neutral buffered formalin for 24 hr. colon tissues that were fixed in formalin were removed and stained with 0.2% Methylene blue (0.2g in 100ml distill water) for 5 min then briefly rinsed with distilled water. They were then placed on a microscopic slide and observed under a light microscope at 40X magnification. ACF were distinguished by their slit like opening, darkly stained, size and pericryptal zone, and slight elevation compared to normal crypts.[12]

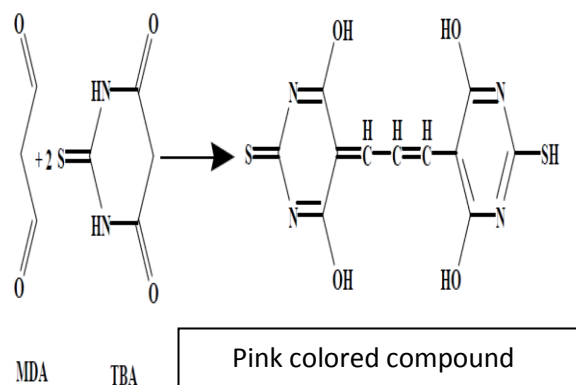
**2% Tissue Homogenate:** 100 mg of tissue was weighed appropriately and homogenized in 5ml of 0.1M phosphate buffer P<sup>H</sup> 8.0 with Remi motor at a speed of 2500 rpm for 2 minute in ice cold surrounding environment.

**Estimation of MDA Levels:**

TBARS level in tissue is a measure of lipid per oxidation. It was measured in colon tissue homogenate using the method described by Ohkawa et al [13]

**Principle:**

The test is based on the fact that the MDA formed as a result of lipid peroxidation react with TBA (Figure 2) and produce TBARS ([TBA]<sub>2</sub>-MDA adduct), a pink chromogen.



**Fig: 2 Reaction of TBA with MDA to Yield Pink Colored Compound**

**Procedure:**

500µl of supernatant of 2% tissue homogenate in 0.15mol/L Kcl was mixed with 200µl 8.1% SDS and then incubated at room temperature for 5 min Add 1.5ml of 20% acetic acid (pH 3.5) & 1.5ml 0.8% thiobarbituric acid. The reaction mixture was heated at 95°C for 90 minutes.

Cool the mixture and add 1ml of distilled water & 5ml butanol /pyridine (15:1) solution under agitation using a vortex. This solution was centrifuged at 1000xg for 15min & the resultant colored layer was separated and measured at 532nm using spectrophotometer.

**Calculations:**

$$\text{The concentration of MDA} = \frac{\text{Absorbance}}{L} * \frac{1}{\epsilon} * D$$

L: Light path (cm)

ε : Extinction coefficient (1.56 x 10<sup>5</sup> M<sup>-1</sup> .Cm<sup>-1</sup>)

D: Dilution factor

**Units:** nmol of MDA/ mg protein.

**Measurement of Catalase Activity:**

Catalase activity of Colonic tissue homogenate was measured by the method of Aebi [14]

**Principle:**

Catalase enzyme readily decomposes the H<sub>2</sub>O<sub>2</sub> into oxygen and water molecule. The decrease in the absorbance due to hydrogen peroxide H<sub>2</sub>O<sub>2</sub> reflects the consumption by the enzymethus assess the concentration of enzyme.

**Procedure:** 0.1ml of supernatant of 2% tissue homogenate in 0.1M phosphate buffer pH 7.4 was added to cuvette containing 1.9ml of 50mM phosphate buffer (pH 7.0)

Reaction was started by the addition of 1.0ml of freshly prepared 30mM H<sub>2</sub>O<sub>2</sub>.

The absorbance was read at 15 and 30 seconds at 240 nm.

**Calculations:**

$$\text{Activity of Catalase (K/ml)} = \frac{Vt}{Vs} * \frac{2.303}{\Delta t} * \log \frac{A1}{A2} * 60$$

Δt: (time 2 - time 1) = 15 seconds.

A<sub>1</sub>: First absorbance at 15 seconds.

A<sub>2</sub>: Second absorbance at 30 seconds.

V<sub>t</sub>: Total volume = 3 ml.

V<sub>s</sub>: Sample volume = 2 ml.

K: First order rate constant. It expresses the activity of Catalase.

**Units:** K/ml = Specific activity.

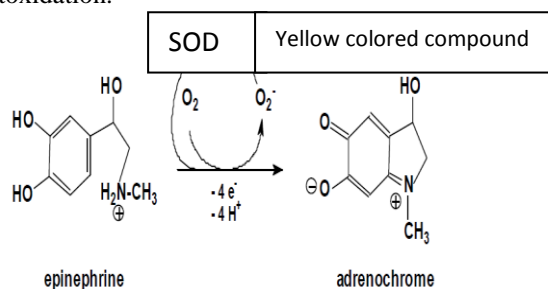
**Measurement of SOD Activity:**

SOD level was measured in colonic tissue homogenate using the method described by Misra et al (1972) [15]

**Principle:**

The oxidation of epinephrine was followed in terms of the production of adrenochrome, which exhibits an absorption maximum at 480nm (Figure 3). Adrenochrome is unstable at pH 10.2 in air. Epinephrine is quite stable in acid solutions but gets auto-oxidized with rapidly increase in the pH . At pH 10.2 the linear rate of accumulation of adrenochrome in solutions of epinephrine is a complex function of the concentration of epinephrine. The autoxidation of epinephrine at pH 10.2 is strongly inhibited by superoxide

dismutase. EDTA increases the sensitivity of epinephrine to superoxide dismutase, at the same time has no effect on the maximum degree of inhibition which could be achieved. It also chelate with heavy metals that might affect the enzyme activity in the reaction mixture. The sensitivity of the oxidation of epinephrine towards the inhibition by superoxide dismutase, decreases with pH and become zero at pH 8.5 and below 100% autoxidation of epinephrine to adrenochrome was performed without enzyme and used as control. The enzyme activity is expressed as unit's mg-1 protein. One unit of enzyme activity is defined as the enzyme required causing 50% inhibition of epinephrine autoxidation.



**Fig 3: Oxidation of Epinephrine and Inhibition by SOD.**

**Procedure:**

0.5ml of supernatant (2% tissue homogenate in 0.1 M phosphate buffer, pH 7.4) was taken.  
1.5ml of carbonate buffer (pH 10.2) was added.  
0.5ml of 0.1mM EDTA was added.  
0.4ml of epinephrine was added just before taking the optical density.  
Measure the optical density spectro photometrically at 480 nm.

**Calculations:**

$A = \epsilon bc$      $A =$  absorbance     $\epsilon =$  Molar absorptivity coefficient ( $4020 \text{ M}^{-1} \text{ Cm}^{-1}$ )  
 $b =$  path length (cm)  
 $c =$  concentration

**Units:**  $\mu\text{mol/mg tissue}$

**Measurement of Reduced Glutathione (GSH)**

**Levels:**

Reduced glutathione (GSH) level was measured in colonic tissue using the method described by Ellman (1959) [16-17]

**Principle:** 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB) is a disulfide chromogen that is readily

reduced by sulfahydryl group of GSH to an intensely yellow compound. The absorbance of the reduced chromogen is measured at 412 nm, directly proportional to the GSH concentration as in the following (Figure 4)

**Procedure:**

Tissue was taken and homogenized in 0.1M phosphate buffer pH 7.4.  
The homogenate was added with equal volume of 20% Trichloro acetic acid containing 1mM EDTA to precipitate the tissue proteins.  
Mixture was allowed to stand for 5 minutes.  
Centrifugation was done at 200 rpm for 10 minutes.

Supernatant (200  $\mu\text{l}$ ) was taken in fresh tubes  
1.8ml of the Ellman's reagent (0.1mM) was prepared in 0.3M phosphate buffer with 1% sodium citrate solution.

The test tubes were made up to 2 ml.

After completion of total reaction, solution was read at 412 nm against blank

**Calculations:**

The concentration of GSH =  $\frac{\text{Absorbance}}{L} * \frac{1}{\epsilon} * D$

L: Light bath (cm)

$\epsilon$  : Extinction coefficient ( $14150 \text{ M}^{-1} . \text{Cm}^{-1}$ )

D: Dilution factor.

**Units:**  $\mu\text{mol / mg protein}$ .

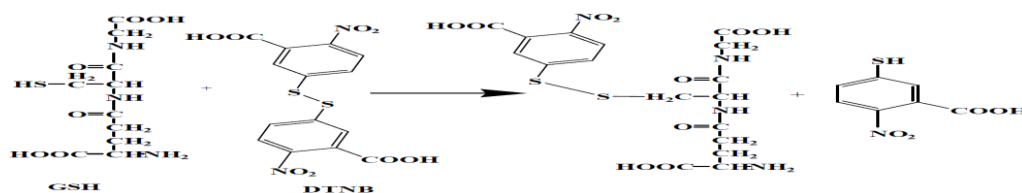
**Statistical Analysis:**

Data expressed as Mean  $\pm$  SEM were analyzed by one way analysis of variance (ANOVA) followed by Turkey's test as a post hoc by using Graph Pad prism software

**RESULTS :**

**Aberrant Crypt Foci:**

Aberrant Crypt Foci formation is an indicator of colon carcinogenesis. ACF are clusters of tube like glands distinguished by their slit like opening, darkly stained, and slightly elevated compared to normal crypts. Table 1 summarizes the effect of *Cassia absus* on ACF formation. In the disease control group the number of aberrant crypts will be more due to induction of colon carcinogenesis by DMH. In the group treated with *Cassia absus* at 200 mg/kg, the number of aberrant crypts has been reduced to some extent, where as in the group treated with *Cassia absus* 400 mg/kg, the number of Aberrant Crypts are significantly reduced when compared with disease and standard group.



**Fig 4: Reaction of DTNB with GSH Yielding Yellow Colored Compound**

**Table 1: Effect of *Cassia absus* (200 mg/kg and 400 mg/kg) on DMH-induced colonic ACF formation in Swiss mice**

Groups	Number of ACF with			
	1 crypt	2 crypts	3 crypts	≥ 4 crypts
Disease control	67.34±1.570	36.9±2.670	42.3±1.05	30.66±1.570
Standard group	29.77±0.499 <sup>b</sup>	7.90±9.90 <sup>a</sup>	10.8±1.679 <sup>a</sup>	19.23±0.499 <sup>b</sup>
Cassia absus (200 mg/kg)	40.80±2.690 <sup>b</sup>	15.78±2.570 <sup>b</sup>	24.9±2.320 <sup>b</sup>	19.80±2.690 <sup>b</sup>
Cassia absus (400 mg/kg)	10.9±1.560 <sup>a</sup>	7.0±0.750 <sup>a</sup>	3.2±0.897 <sup>a</sup>	4.2±1.560 <sup>a</sup>

Data was expressed as mean ± SEM (n=4). Data analyzed by one way analysis of variance (ANNOVA) followed by Tukey's test for the comparison of means ; <sup>a</sup>p<0.001, <sup>b</sup>p<0.01 compared to disease control group



Fig :5 a

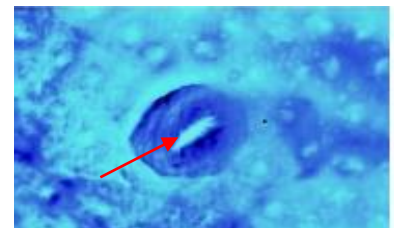


Fig :5 b

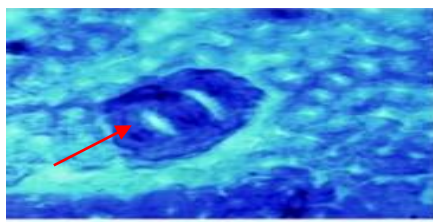


Fig : 5 c

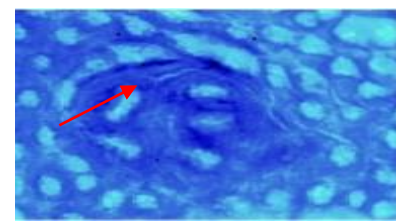


Fig :5 d

**Fig5a:** Represents normal colon, **fig5b:** Represents colonic ACF with 1 crypt, **fig5c:** Represents colonic ACF with 2 crypts , **fig5 d:** Represents colonic ACF with ≥ 4 crypts.

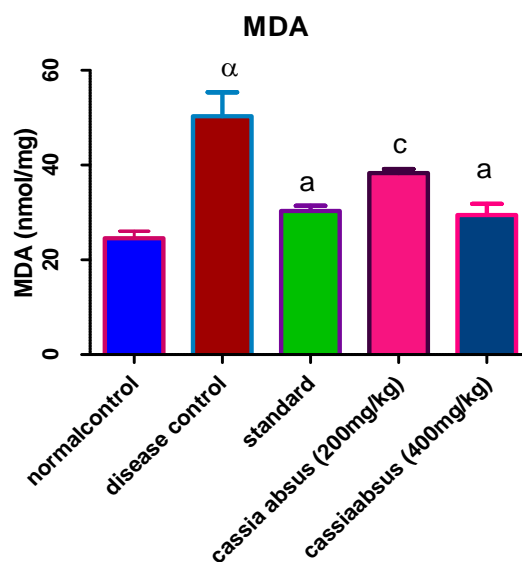
#### Colonic Malondialdehyde (MDA) levels:

Lipid per oxidation is a consequence of oxidative stress. MDA is an index of lipid per oxidation. High MDA levels indicate high extent of lipid per oxidation in the tissue due to oxidative stress. Table 2 summarizes the data on the effect of *Cassia absus* on MDA levels in colon of mice. In normal control group, average MDA value is 24.58 ± 3.314. In disease control group average MDA levels are

raised to 47.326 ± 1.57 due to induction of oxidative stress by DMH. In the groups treated with *Cassia absus* (200 mg/kg), the average MDA levels are decreased to 36.90 ± 3.298. In the groups treated with *Cassia absus* (400 mg/kg), the average MDA levels are decreased to 26.62 ± 1.482, which is considered as statistically significant (p<0.001), when compared with normal and standard groups

Table 2: Colonic MDA Levels of Different Groups in Mice

S. no.	Normal group (nmol/mg)	Disease group (nmol/mg)	Standard group (nmol/mg)	<i>Cassia absus</i> (200mg/kg) (nmol/mg)	<i>Cassia absus</i> (400 mg/kg) (nmol/mg)
1	26.47	25.36	32.69	39.60	33.58
2	27.56	52.62	27.11	37.80	20.67
3	20.64	57.82	31.34	40.42	29.70
4	26.92	56.41	32.17	38.45	33.20
5	21.34	54.42	28.33	35.25	30.33
<b>Mean±Sem</b>	24.58±3.31	47.32±1.57	30.32±1.10	36.90±3.29	26.62±1.48

Fig 6: Modulatory Effects of *Cassia absus* on MDA Levels in Colon:

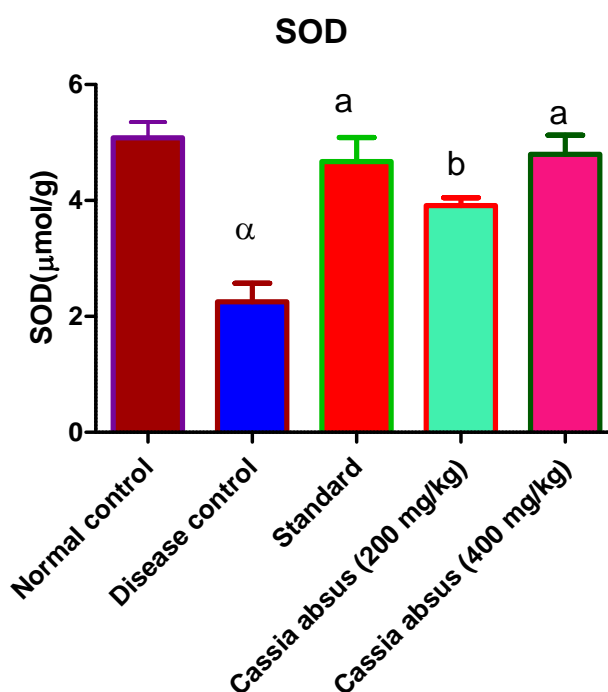
Data was expressed as Mean  $\pm$  SEM (n=6). Data analyzed by one way analysis of variance (ANNOVA) followed by Tukey's test for the comparison of means. <sup>a</sup>p<0.001, compared to normal control group; <sup>a</sup>p<0.001, <sup>c</sup>p<0.01 compared to Disease control group.

**Colonic Superoxide Dismutase levels:** Superoxide dismutase is an important antioxidant defense enzyme system present in all cells exposed to oxygen. SOD catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. Oxidative stress results in depletion of SOD levels

in the colonic tissue. Table 3 summarizes the data on the effect of a *Cassia absus* on SOD levels in colon of mice. In normal control group, average SOD value is  $5.032 \pm 0.230$ . In disease control group average SOD levels are decreased to  $2.253 \pm 0.317$  due to induction of oxidative stress by DMH. In the groups treated with *Cassia absus* (200 mg/kg), the average SOD levels are increased to  $4.67 \pm 0.412$ . In the groups treated with *Cassia absus* (400 mg/kg), the average SOD levels are increased to  $4.807 \pm 0.323$ , which is considered as statistically significant (p<0.001), when compared with normal and standard groups.

Table 3: Colonic Levels of SOD in Different Groups of Mice

S.no.	Normal group ( $\mu\text{mol/g}$ )	Disease group ( $\mu\text{mol/g}$ )	Standard group ( $\mu\text{mol/g}$ ).	<i>Cassiaabsus</i> (200mg/kg) ( $\mu\text{mol/g}$ )	<i>Cassia absus</i> (400 mg/kg) ( $\mu\text{mol/g}$ )
1	4.45	1.24	4.22	3.45	3.80
2	5.37	3.23	4.72	3.78	4.52
3	4.90	1.99	4.97	3.90	5.37
4	4.72	1.49	3.98	4.23	5.67
5	5.97	2.73	3.48	4.12	4.63
Mean $\pm$ Sem	5.03 $\pm$ 0.23	2.25 $\pm$ 0.31	4.78 $\pm$ 0.41	4.67 $\pm$ 0.41	4.80 $\pm$ 0.32

Fig 7: Modulatory Effects of *Cassia absus* on SOD Levels in Colon:

Data was expressed as Mean  $\pm$  SEM (n=6). Data analyzed by one way analysis of variance (ANNOVA) followed by Tukey's test for the comparison of means. <sup>a</sup>p<0.001 to normal control group; <sup>a</sup>p<0.001, <sup>b</sup>p<0.01, compared to Disease control group.

#### Colonic Catalase Levels:

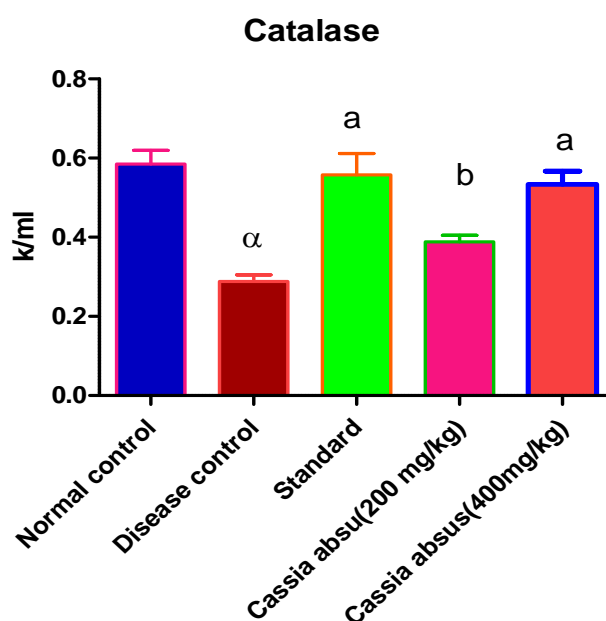
Catalase is an antioxidant enzyme system present in all living cells exposed to oxygen. Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen and thus protects the cell from oxidative damage. Oxidative stress may result in decrease in Catalase levels in the colonic tissue. Table 4 summarizes the data on the effect of *Cassia absus* on Catalase levels in colon of mice. In normal control group, average Catalase value is

0.578  $\pm$  0.036. In disease control group average Catalase levels are decreased to 0.286  $\pm$  0.27392 due to induction of oxidative stress by DMH. In the groups treated with *Cassia absus* (200 mg/kg), the average Catalase levels are increased to 0.3882  $\pm$  0.016. In the groups treated with *Cassia absus* (400 mg/kg), the average Catalase levels are increased to 0.534  $\pm$  0.033, which is considered as statistically significant (p<0.001), when compared with normal and standard groups



Table 4: Colonic levels of Catalase in Different Groups of Mice

S. NO	Normal group(k/ml)	Disease Group(k/ml)	Standard group(k/ml)	<i>Cassiaabsus</i> (200mg/kg) (k/ml)	<i>Cassiaabsus</i> (400mg/kg) (k/ml)
1	0.574	0.286	0.680	0.336	0.432
2	0.680	0.268	0.372	0.398	0.540
3	0.642	0.349	0.690	0.414	0.598
4	0.486	0.252	0.517	0.368	0.610
5	0.517	0.292	0.428	0.427	0.488
<b>Mean±Sem</b>	0.57±0.03	0.28±0.0274	0.55±0.05	0.38±0.016	0.534±0.033

**Fig 8: Modulatory Effects of *Cassia absus* on Catalase Levels in Colon:**

Data was expressed as Mean  $\pm$  SEM (n=6). Data analyzed by one way analysis of variance (ANNOVA) followed by Tukey's test for the comparison of means.

<sup>a</sup>p<0.001, compared to normal control group; <sup>a</sup>p<0.001, <sup>b</sup>p<0.01 compared to Disease control group.

**Colonic GSH levels:** GSH is a free radical scavenging system, acts by preventing damage to cellular components from reactive oxygen species. Increased oxidative stress may cause depletion in GSH levels in the colonic tissue. Table 5 summarizes the data on the effect of *Cassia absus* on GSH levels in colon of mice. In normal control group, average GSH value is  $4.978 \pm 0.243$ . In disease control group average GSH levels are

decreased to  $2.64 \pm 0.249$  due to induction of oxidative stress by DMH. In the groups treated with *Cassia absus* (200 mg/kg), the average GSH levels are increased to  $4.104 \pm 0.107$ . In the groups treated with *Cassia absus* (400 mg/kg), the average GSH levels are increased to  $4.57 \pm 0.153$ , which is considered as statistically significant (p<0.001), when compared with normal and standard groups.

Table 5: Colonic Levels of GSH in Different Groups of Mice.

S. No.	Normal group ( $\mu\text{mol/g}$ )	Disease Group ( $\mu\text{mol/g}$ )	Standard group ( $\mu\text{mol/g}$ )	<i>Cassia absus</i> (200 mg/kg) ( $\mu\text{mol/g}$ )	<i>Cassia absus</i> (400 mg/kg) ( $\mu\text{mol/g}$ )
1	4.38	1.83	4.02	4.09	3.59
2	4.94	2.96	3.53	3.68	3.78
3	5.58	2.68	4.31	4.39	4.02
4	4.59	3.18	3.67	3.83	3.94
5	4.16	2.85	3.46	3.45	4.80
Mean $\pm$ Sem	4.978 $\pm$ 0.24	3.84 $\pm$ 0.107	4.34 $\pm$ 0.132	4.10 $\pm$ 0.107	4.57 $\pm$ 0.153

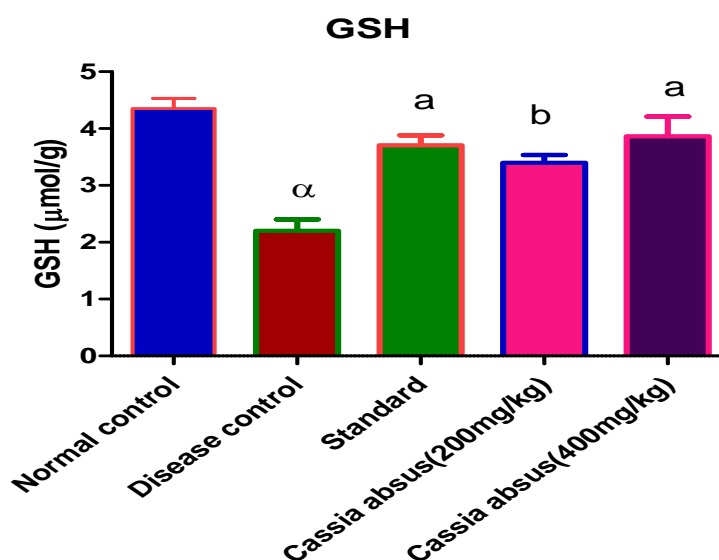


Fig 9: Modulatory effects of cassia absus on GSH levels in colon:

Table 6: Mean Colonic Levels of Oxidative Parameters like MDA, SOD, Catalase, and GSH on Different Groups of Mice

Groups	BIO-CHEMICAL PARAMETERS			
	MDA ( $\eta\text{mol/mg}$ )	SOD ( $\mu\text{mol/g}$ )	Catalase (K/ml)	GSH ( $\mu\text{mol/g}$ )
Normal control	24.58 $\pm$ 1.482	5.032 $\pm$ 0.232	0.588 $\pm$ 0.093	4.73 $\pm$ 0.555
Disease control	47.92 $\pm$ 5.384 <sup>α</sup>	2.13 $\pm$ 0.317 <sup>α</sup>	0.276 $\pm$ 0.052 <sup>α</sup>	2.70 $\pm$ 0.519 <sup>α</sup>
Standard control	30.32 $\pm$ 1.103 <sup>a</sup>	4.67 $\pm$ 0.492 <sup>a</sup>	0.53 $\pm$ 0.146 <sup>a</sup>	3.79 $\pm$ 0.358 <sup>a</sup>
Cassiaabsus(200 mg/kg)	36.90 $\pm$ 4.298 <sup>c</sup>	3.911 $\pm$ 0.51 <sup>b</sup>	0.492 $\pm$ 0.05 <sup>b</sup>	4.54 $\pm$ 0.394 <sup>b</sup>
Cassiaabsus(400 mg/kg)	30.62 $\pm$ 0.972 <sup>a</sup>	4.807 $\pm$ 0.720 <sup>a</sup>	0.589 $\pm$ 0.137 <sup>a</sup>	4.77 $\pm$ 0.391 <sup>a</sup>

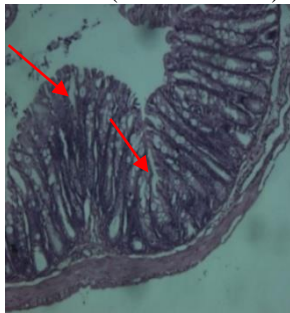
Data was expressed as Mean $\pm$ Sem (n=6). Data analyzed by one way analysis of variance (ANNOVA) followed by Tukey's test for the comparison of means. <sup>a</sup>p<0.001 to normal control group; <sup>a</sup>p<0.001, <sup>b</sup>p<0.01, compared to Disease control group.

Data was expressed as mean  $\pm$  SEM (n=4). Data analyzed by one way analysis of variance (ANNOVA) followed by Tukey's test for the comparison of means; <sup>a</sup>p<0.001, compared to Normal control group, <sup>a</sup>p<0.001, <sup>b</sup>p<0.01, <sup>c</sup>p<0.05 compared to Disease control group.

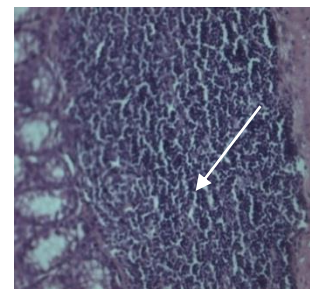
#### Histopathological examinations:

Histopathological findings of colon in normal mice revealed normal mucosal (Red arrows), sub mucosal is normal (White arrows) with regular

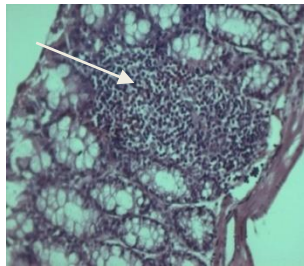
crypts with no inflammation (Fig: 10a). The group treated with DMH, colonic tissue has shown inflammatory cells in the sub mucosal layer (white arrows) and the colonic tissue exhibited dysplasia. In the group treated with standard drug, 5-fluorouracil, there is moderate inflammation in the sub mucosa (white arrows), and moderate dysplasia. The group treated with *Cassia absus* (200 mg/kg) has shown moderate sub mucosal inflammation (white arrows) and moderate dysplasia. The group treated with *Cassia absus* (400 mg/kg) has shown normal mucosa (red arrows) and normal sub mucosal layers (white arrows) with no inflammation and dysplasia. Mice treated with *Cassia absus* (400 mg/kg) exhibited normal histology as that of normal control group.



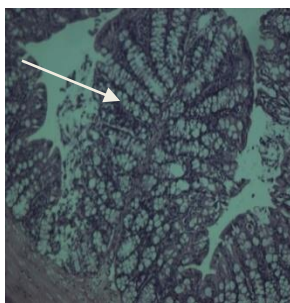
**Fig10 a: Normal colon**



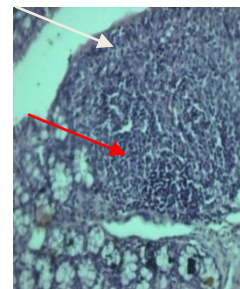
**Fig 10b: Diseased colon**



**Fig10 c: Standard drug**



**Fig10d :Cassia absus(200 mg/kg)**



**Fig 10e: Cassia absus(400 mg/kg)**

**Fig 10a:** In normal group the mucosa ( red arrows) is normal and submucosa (white arrows) is normal,

**Fig 10 b:** In disease group there is submucosal inflammation (white arrows),

**Fig10c:** In standard group there is moderate submucosal inflammation (white arrow)

**Fig 10d:** In Cassia absus 200mg/kg group there is moderate inflammation (white arrow),

**Fig 10e:** In Cassia absus 400 mg/kg group mucosal (red arrows) and submucosal layer (white arrows) is normal.

**DISCUSSION:**

The present study demonstrated that administration of *Cassia absus* significantly ameliorated DMH induced colon cancer in mice evidenced by decreased ACF formation and altered oxidative parameters in a dose dependent manner. A current upsurge in classifying natural products as Cancer chemo preventive agents is gaining much attention of many investigators because natural products like fruits, vegetables, medicinal plants, and herbs have many pharmacological properties and have potential to fight against numerous human diseases including cancer associated with oxidative stress. Cancer chemoprevention is defined as the prevention, inhibition, or reversal of carcinogenesis by administration of one or more chemical entities, either as individual drugs or as naturally occurring constituents of the diet. *Cassia absus* have been reported to possess chemo preventive properties against various tumors.

The earliest identifiable morphological changes in colonic mucosa in the chronology of cancer developments are Aberrant Crypts (AC). These Aberrant Crypts can be identified by their increased size, thicker epithelial lining, and increased pericryptal zone. In more advanced stage of carcinogenesis, the multiplicity of AC's within an ACF increases and correlates with the incidence of colorectal adenomas. ACF formation is known as putative indicator of colon carcinogenesis and efficacy of anti carcinogenic effects. In fact, ACF are considered as gold standard of colon carcinogenesis markers. In agreement with previous reports, it was observed that DMH showed significantly high number of ACs, ACF and Crypt multiplicity. Protective effect of *Cassia absus* was evidenced by decreased in total number of AC per mice and decreased number of AC per focus.

DMH is a Procarcinogen that undergoes metabolic activation in the liver to produce active electrophilic carbonium ions which in turn through several process is known to elicit oxidative stress. Reactive oxygen species, due to their higher reactivity are thus potentially toxic, mutagenic or carcinogenic. Increased free radical activity, including enhanced lipid per oxidation is considered to play a major role in the pathogenesis of cancer. There is considerable evidence to indicate that the severity of oxidative stress in carcinogenesis is associated with decreased antioxidant defence. DMH also act as alkylating agent which alkylates guanine at O6 position which is considered to be an important miscoding lesion leading to G-A transition with a important role in mutagenesis and carcinogenesis. Lipid Per oxidation is a consequence of oxidative stress and remarkable elevation in the level of Malondialdehyde (MDA) a lipid Peroxidation product, was observed after treatment with DMH. In the present study, DMH treated mice showed a

remarkable Increase in the level in the level of MDA and *Cassia absus* significantly attenuated its level in colonic tissues.

Superoxide dismutase (SOD) and Catalase (CAT) are two important enzymatic anti oxidants that act against toxic oxygen free radicals such as superoxide ( $O_2^-$ ) and hydroxyl (OH) ions in biological systems. They are considered as primary anti oxidant enzymes since they are involved in the direct elimination of the reactive oxygen species. GSH, a tripeptide, plays a important role in the detoxification of many environmental carcinogens and free radicals. [11] GSH acts directly as a free radical scavenger by neutralizing hydroxyl radical, restores damaged molecules by hydrogen donation, reduces peroxides and maintains protein thiols in the reduced state. GSH and its dependent enzymes work in concert with other antioxidants and anti oxidant enzymes to protect cells against ROS.

Our data suggest that the activity of SOD, CAT and GSH were decreased in DMH alone treated mice. The depleted levels of these enzymes on DMH administration suggested that these may be involved in detoxicated in detoxification and possibly repair mechanism in colonic mucosa. Supplementation of mice with *Cassia absus* to scavenge resulted in enhanced activity of these enzymes which may be due to the ability of *Cassia absus* to scavenge free radicals and toxic carcinogenic metabolites, thus sparing the endogenous anti-oxidant. It was proven that low levels of SOD, CAT and GSH activity in pre cancerous tissues promoted growth of cancer and its infiltration into surrounding tissues which was important for invasion and metastasis. The partial restoration of these enzyme levels on *Cassia absus* supplementation reflect a favorable balance between potentially harmful oxidants and natural antioxidants, which may be contributed to the chemo preventive effect of *Cassia absus*.

Histopathological studies revealed inflammation, severe dysplasia and focal congestion in muscularis mucosa and sub mucosal layers in mice treated with only DMH. Histopathological evaluation of colonic tissues of mice treated with *Cassia absus* showed suppressed inflammatory responses in the colon by decreasing the intense infiltration of the inflammatory cells in the mucosal and sub mucosal layers and also exhibited mild dysplasia with very mild focal congestion.

**SUMMARY AND CONCLUSION:**

**Summary:** In present study, administration of 1,2 Di Methyl Hydrazine developed Colon cancer is characterized by significant increase in the colonic MDA levels and decreased anti oxidant status and altered Histoarchitectural of Colon. Treatment with

*Cassia absus* (200mg/kg and 400mg/kg) restored Histoarchitectural and also improved the anti oxidant enzyme status and decreased MDA levels.

#### CONCLUSION:

In conclusion, the present study suggests that treatment with *Cassia absus* significantly ameliorated DMH induced colon cancer probably due to their anti oxidant and anti tumor properties. Thus these findings may open novel prospective in cancer chemoprevention.

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