PHARMACOLOGICAL EVALUATION OF *SESBANIA GRANDIFLORA* FOR ANTICOLON CANCER ACTIVITY IN 1, 2 DIMETHYLHYDRAZINE INDUCED COLON CANCER

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ARTICLE INFO	ABSTRACT
Key words: Aberrant crypt foci (ACF); Apoptosis; 1, 2-Dimethylhydrazine (DMH); Ethanolic extract of *Sesbania grandifloria*	Anticancer activity is the effect of natural and synthetic or biological and chemical agents to reverse, suppress or prevent carcinogenic progression. Several synthetic agents are used to cure the disease but they have their toxicity and hence the research is going on to investigate the plant derived chemotherapeutic agents. Therefore an attempt has been made for effective medication to treat cancer.The plant *Sesbania grandiflora* (Fabaceae) is commonly known as Sesbania and agathi in ayurvedic system of medicine. The plant has various uses in folk and traditional medicines for headache, swellings, anemia, bronchitis, pains, liver disorders and tumors. **Aim of the study:** Pharmacological Evaluation Of *Sesbanina Grandiflora* For Anticolon Cancer Activity In 1, 2 Dimethylhydraine Induced Colon Cancer. **Materials and methods:** Anticolon cancer activity in 1, 2 dimethylhydrazine induced colon cancer in rats. Male albino wistar rats were randomly divided into three groups. Group 1 served as control, received gum acacia and group 2 received 30mg/kg b.wt 1, 2 dimethylhydrazine (DMH) intraperitoneally for two weeks.Group 3 receieved 5-FU 20mg/kg b.wt for two weeks. Group 4 received ethanolic extract of *Aegle marmelos* at oral dose of 400mg/kg b.wt every day. Until the end of whole experimental period of 30 days. **Results:** *Sesbaina grandiflora* administration significantly reduced ACF number (p<0.001), increased the weight gain (p<0.05), apoptotic index (p<0.001) compared to DMH group. The histological alterations induced by DMH were also significantly improved. **Conclusion:** Present results confirmed that the *Sesbaina grandiflora* at a dose of 400mg/kg body weight can significantly reduces the formation of aberrant crypt foci (ACF) and number of aberrant crypts and improved histopathological changes in colon cancer bearing rat.

INTRODUCTION:

Cancer is the uncontrolled growth and spread of abnormal cells, associated with dysregulation of apoptosis, a programmed cell death. Most of the current anticancer drugs are derived from plant sources, which act through different pathways converging ultimately into activation of apoptosis in cancer cells leading to cell cytotoxicity (Mishra *et al.,* 2005). According to World Health Organization, more than 10 million new cases of cancer are diagnosed every year, and the statistical trends indicate that this number would double by 2020 (Mignogna MD *et al.,* 2004).Colon cancer rate in India is lower than the western countries, but is increasing with increasing migration of rural population to the cities, increase in life expectancy and changes in life style (Srikhande *et al.*, 2007). The metabolism of 1, 2-dimethylhydrazine (DMH) in the liver

quantitatively predominates over organ-specific metabolism. Both proximate and ultimate metabolites are formed in the liver that can be transported to the colon resulting in DNA alkylation in the colonocytes. It is believed that alkylation of specific sites in DNA leads to promutagenic events which may result in tumour initiation (Harbach *et al.,* 1981). The metabolic activation of DMH follows the following steps (i) hydroxylation of DMH to methyl azoxy methanol, which occurs predominantly in the liver, and to a limited degree in the colonic mucosa probably via a cytochrome P450-dependent pathway and (ii) oxidation of methylazoxymethanol to methylazoxyformaldehyde, which is catalyzed by the microsomal/cytosolic enzymes of the liver and colon in these tissues. Subsequently the unstable compound, methylazomethanol, readily yields methyldiazonium ion, which can modulate the enzymatic and non-enzymatic processes in the liver and colon (Tanaka *et al.,* 1997). Both normal and tumor cells metabolize 5-FU to 5-fluoro-2'- deoxyuridine monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP). These metabolites cause cell injury by two different mechanisms. First, FdUMP and the folate cofactor N5-10-methylene-tetrahydrofolate bind to thymidylate synthase (TS) to form a covalently bound ternary complex. This binding inhibits the formation of thymidylate from 2'-deoxyuridylate. Thymidylate is the necessary precursor of thymidine triphosphate, which is essential for the synthesis of DNA, so that a deficiency of this compound can inhibit cell division. Second, nuclear transcriptional enzymes can mistakenly incorporate FUTP in place of uridine triphosphate (UTP) during the synthesis of RNA. This metabolic error can interfere with RNA processing and protein synthesis.

USES IN TRADITIONAL MEDICINE AND REPORTED ACTIVITIES:

It is widely used in Indian traditional medicine for treatment of a broad spectrum of diseases including leprosy, gout, rheumatism, tumor, liver disorders (Joshi 2000). The fruits are bitter and acrid, laxative, cures "tridosha" fever, pain, bronchitis, anaemia, tumours, improves taste (Ayurveda). Several reports suggested that the ethanolic extract of the bark of *Sesbania grandiflora* prevented acute gastric injury in rats. The leaf juice of *Sesbania grandiflora* showed significant antiurolithiatic activity (Ojha JK and Dwivedi KN 1996). *In vivo* studies, *Sesbania grandiflora* administration showed potential anticancer (Doddola S 2008), anxiolytic (Laladhas KP 2009), hepatoprotective in rats (Kasture *et al.*, 2002), antimicrobial, analgesic, antipyretic activity was evaluated. *Sesbania grandiflora* are richest source of amino acid, minerals and antioxidants vitamins (Govindan and Shanmugasundaran 1987) It also has anxiolytic and anticonvulsive, (Kasture *et al.,* 2002) anti inflammatory, analgesic and antipyretic activity (Tamboli 2000) Besides *S. grandiflora* is mentioned as a potent antidote for tobacco and smoking related diseases. *Sesbania grandiflora* has hypolipidemic property on cigarette smoke exposed rats (Ramesh and Hazeena begum 2006).

MATERIALS AND METHODS

Materials: 1,2dimethylhydrazine were purchased from sigma-aldrich company, Bangalore. Methylene blue from Sico Research Laboratories private limited, 5-Florouracil was obtained from Ranbaxy Laboratories, limited India and Haemotoxylin from Merck specialties private limited, Mumbai. All the solvents and other chemicals were procured from SS pharma, Hanamkonda and they were of analytical grade quality. Wistar albino rats were procured from Mahaveer enterprises, Hyderabad.

Collection and identification of plant material: For the present investigation, the pods of *Sesbaina grandiflora* were collected from near Laknavaram lake, Warangal District, Andhra Pradesh, India and authenticated by expert taxonomist Dr. E. Narasimha Murthy.

Acute toxicity studies: An acute toxicity study procedure was followed by using OECD guidelines (Organisation of Economic Cooperation and Development) 423 (Acute Toxic Class Method). Doses (5, 50, 500, 2000 mg/kg b.wt and the results allow a substance to be ranked and classified according to the Globally Harmonised System (GHS) for the classification of the chemical while causes acute toxicity. Dose was administered to the Mice, which were fasted overnight with water ab libitum, food were withheld for further 3-4hrs. After administration of drugs and observed for another 14days.The animals were

observed for behavioural, neurological and autonomic profiles.

Preparation of test drug: EESG was suspended in distilled water and each rat received a daily 1ml as suspension at a dose of 400mg/kg body weight orally by oral gavage throughout the experiment period

Animals and Experimental design: A total of 24 male albino Wistar rats were obtained from Mahaveer Enterprises (Reg no.146/1999CPCSEA) Hyderabad, Andhra Pradesh. The animals were kept in polypropylene cages (4 per cage) and fed standard pellet diet for 1 week. Thereafter, the animals were randomly divided into four groups each containing 6 rats and maintained under controlled conditions of temperature (24 ± 2 °C), humidity (50 ± 10%), and 12-h light/dark cycle and tap water was provided *ad libitum.* The animals were cared for incompliance with the principles and guidance of institutional animal ethical committee (1047/ac/07/CPCSEA). Animals were quarantined and acclimatized to laboratory conditions for 1 week prior to study initiation.

Rats were randomly assorted to 4 experimental groups (16 rats/group): Group 1 served as normal control, which received vehicle (distilled water), group 2 rats served as disease control were given Intraperitoneal injections of DMH twice a week for 2 consecutive weeks at 30 mg/kg b.wt., Group 3 is given with 5-Fluorouracil (20mg/kg body weight) intraperitoneally for 2 weeks, in addition to this group 4 rats were also given EESG at a dose of 400 mg/kg body weight daily for total 30 days of study period by oral route. Body weights (every week) were recorded, and at the starting and end of experimental period blood samples were taken and various blood parameters were recorded. At the end of the study period all the rats were sacrificed under ether anaesthesia and cervical dislocation.

Tumour induction: 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 (Aranganathan *et al.,* 2009).The rats were give intraperitoneal injection of DMH twice a week for two weeks at a dose of 30 mg/kg body weight (Aroch *et al.*, 2010).

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Body weight changes: The body weight changes of the control, DMH and EESG treated rats were measured throughout the study. The rats were weighed at the beginning of the experiment and then subsequently once week and finally before sacrifice (Arangantha *et al.*, 2003).

Determination of aberrant crypt foci: At the end of study, their colons were removed and flushed with an isotonic saline solution (0.1 M, pH 7.2). They were then opened longitudinally, cut into two parts of equal length and labelled as the proximal, distal segments (Aroch *et al.*, 2010).The colon were secured and fixed in a try containing 10% buffered formalin overnight. Each segment was stained with 0.2% Methylene blue solution for 2 min (Takefumi *et al.*, 2006). The segments were examined using a light microscope at a low magnification to score the total number of crypts per focus. ACF were distinguished from normal crypts by their thicker, darker- stained, raised walls with elongation slit-like lumens and significantly increased distance from the lamina to basal surface of cells (Aranganathan *et al.*, 2009).

Apoptosis measurement in colonic mucosa: Apoptosis evaluation was carried out in paraffin-embedded sections of normal colonic, mucosa and tumours stained with haematoxylin. At least 20 full longitudinal crypt section of normal mucosa were scored at the microscope, determining the prescence of cells in each crypt with the following characteristics of apoptosis: cell shrinkage, loss of normal contact with the adjacent cells of the crypt, chromatin condensation or formation of round or oval nuclear fragments (apoptotic bodies). 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 for the presence of apoptotic cells that were coded. In tumours and colon mucosa, apoptosis was scored by a single observer on coded samples and quantified as apoptotic index (AI)

$$AI = \text{Number of apoptotic cells/cells scored} \times 100$$

Colon crypt pathology: The distal part of the colon that was used in quantification of ACF was washed in 70% ethanol and H_2O to rinse off excessive dye. Colon were embedded in paraffin and sectioned parallel to the mucosa to obtain 4µm thick sections. Theses sections were then stained with Haemotoxylin and eosin. The slides were screened under light microscope. A minimum of 16 ACF with a crypt multiplicity of 3 crypts/foci were selected for assessment (Alrawi *et al.*, 2006).

Haematological evaluation: Before the initiation of the study and immediately before necropsy, blood samples were collected for haematological analysis in vacutainer tubes with 1.5% EDTA and differentially quantified through a coulter T890 for the following leukocyte, erythrocyte and platelets count and haemoglobin determination (Dias *et al.*, 2006).

Individual organ weight: At necropsy, the liver, kidneys, colon, sleep, heart, pancreas, stomach, lungs were removed weighed and relative weight of organs was calculated.

Histopathology of colon: The colon were excised, flushed with saline, cut open longitudinally along the main axis, and then again washed with saline. These colonic sections fixed in 10% buffered formalin for at least 24 h for 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 deparaffinized using xylene and ethanol. These sections stained with haematoxylin and eosin and were observed under light microscope at 10x and 40x magnifications to investigate the histoarchitecture of colonic mucosa (Hamita *et al.*, 2012).

IN VITRO METHODS

1. Measurement of lipid peroxidation: TBARS, a measure of lipid peroxidation was measured. 1 ml of suspension medium was taken from the 10% tissue homogenate. 0.5 ml of 30% trichloroacetic acid (TCA) was added to it followed by 0.5ml of 0.8% thiobarbituric acid (TBA) reagent. The tubes were covered with alumium foil and kept in shaking water bath for 30 mintes; tubes were taken out and kept in ice cold water for 30 minutes. These were centrifuged at 3000 rpm for 15 minutes. The absorbance of the supernatant was read at 540 nm at room temperature against blank. Blank consist of 1 ml distilled water, 0.5 ml 0f 30% TCA and 0.5 ml of 0.8% TBA. TBARS values were expressed as n moles malonaldehyde (MDA)/mg protein (Akhtar *et al.,* 2013).

2. Measurement of reduced GSH: Glutathione was measured according to the method of (Ellman 1959). The equal quantity of homogenate (W/V) and 10% TCA were mixed and centrifuged to separate the proteins. To 0.01 ml of supernatant, 2 ml of phosphate buffer (pH 7.4), 0.5 ml 5, 5-dithiobisnitro benzoic acid (DTNB), and 0.4ml of double distilled water was added. The mixture was vortexed and the absorbance was read at 412 nm within 15 minutes. GSH values were expressed as µ moles GSH mg protein (Akhtar *et al.,* 2013).

3. Measurement of Catalase: Catalase activity was measured by the method of claiborn. A total of 0.1 ml of supernatant was added to curvette containing 1.9 ml of 50 mM phosphate buffer (pH 7). The reaction was started by the addition of 1ml freshly prepared 30mM H_2O_2. The rat of decomposition of H_2O_2 was measured spectrophometerically at 240nm. Catalase values were expressed as n moles H_2O_2 consumed/min/mg protein (Akhtar *et al.,* 2013).

Statistical analysis: Values are given as means ± SEM 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 $p < 0.001$.

RESULTS

1. Acute toxicity study: No adverse effects and no mortality of animals were observed during the period of study, 1st 48 hrs continuously observed for 14days. Up to the dose 4g/kg of extract administered through oral route. Since EESG was found to be non-toxic and a dose of 400mg/kg of extract was selected for studied in experimental animals.

2. Body weight changes: During the experimental period 30days, the carcinogen-exposed rats exhibited a significantly low gain in body weight and low growth rat throughout the experimental period as compared to Group 2 and Group 4. Oral administration of EESG at a dose of 400mg/kg b. Wt resulted in a significant ($p<0.01$) improvement in weight gain relative to treatment with DMH alone.

3. 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 EESG at 400mg/kg b. wt significantly reduced ($P<0.001$) the formation as well as the total number of ACF,AC as compared to rat injected with DMH alone, but there was no reduction in number of aberrant crypts per ACF. No ACF formation was observed in the control.

4. Apoptosis index: The apoptotic 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 the total number of counted cells in a whole colonic crypt. The apoptotic index was significantly ($p<0.05$) increased in group treated with DMH+EESG compared to only DMH treated group.

5. Haematological parameters: Various haematological parameters are compared among the groups of colon cancer. There were significant ($P<0.001$) changes during the study period.

6. Relative weight of organs: Relative weights of various organs are compared among the groups after induction of colon cancer. There was significant ($p<0.001$) reduction in organ weights.

7. Measurement of lipid peroxidation: The TBARS levels measured were found to be significantly increased in the DMH induced rats than in normal rats. The EESG produced significantly reduction in TBARS levels when compared to that of DMH induced colorectal rats. ($p<0.001$)(Table 7) (Fig 6)

8. Measurement of reduced GSH: The colon glutathione levels were estimated in all groups.

Levels of reduced glutathione in DMH induced rats than in normal rats. The extract produced significantly elevated when compared to the EESG group ($p<0.001$) (Table 8) (Fig 7).

9. Measurement of Catalase: The levels of catalase were reduced in DMH induced rats as compared to the normal group. The EESG showed elevation in the levels as compared to DMH group ($p<0.001$) (Table 9) (fig 8)

DISCUSSION

Recently, the use of some herbs has attracted a great deal of attention as one of the alternative cancer therapies from the point of less toxicity and cost benefits. Therefore, an attempt has been made to evaluate the pharmacological evaluation of *Sesbaina grandiflora* for anticolon cancer activity in 1, 2 dimethylhydrazine induced colon cancer, which is commonly used as ayurvedic system of medicine for various purposes. Hydrazine and its derivatives like DMH are such chemicals that are shown to be carcinogenic and mutagenic (Kawanishi *et al.,* 1991). DMH, an alkylating agent, when injected intraperitoneally, is transported to the liver where it undergoes dehydrogenation and is converted to an active carbonium ion through several processes, to be excreted in the bile, where it mediates its carcinogenic activities on the mucosa while passing through the digestive tract (Fiala *et al.,* 1975). Carbonium ions methylate DNA bases, induce point mutations, micronuclei and sister chromatid exchanges leading to colon-specific carcinogenesis (Choudhary *et al.,* 1998).In acute toxicity, no gross behavioural changes and mortality was observed up to a dose level of 400 mg/kg body weight. The LD_{50} value of EESG pods were found very less toxic to the animal. As expected carcinogen (DMH) treated animals in our study showed reduced weight gain compared to other groups. It may be due to decreased food intake. In addition to altered glucose metabolism and increased hepatic gluconeogenesis depletes the energy sources (Gold *et al.,* 1974) leading to a significant weight loss in DMH treated animals.

Table 1: Body weight changes on treatment with ethanolic extract of *Sesbania grandiflora* in DMH induced colon cancer rats on 28th day

Group/treatment	Initial body weight(g)	Final body weight(g)	Weight gain(g)
Control	150±11.18	184.16±12.27	16.66±0.27
DMH	115±8.06	235±13.6[c]	10.00±0.4
DMH+5-FU	140±8.16[a]	202.5±13.88	5.00±6.61
DMH+EESG	153.33±9.86[a]	208.33±8.72	26.67±0.56[c]

Values are mean ± SEM, n=6 in each group, statistically significant [a]***p<0.001,[c]*P<0.05 when compared with disease control (DMH treated) group (One way ANOVA fallowed by Dunnet's test).
EESG=Ethanolic Extract of *Sesbania grandiflora*; DMH=1, 2 Dimethyl hydrazine.

Table 2: Distribution of altered aberrant crypt foci (ACF) category in proximal, distal and total colon of rats exposed to DMH and EESG

Group/treatment	Total no. of AC	Total no of ACF	Crypt/ACF
Proximal colon			
Control	0	0	0
DMH	20.83±2.00	16.16±1.83	2.33±0.21
DMH+5-FU	13.66±1.76[c]	12.83±1.01	2.16±0.47
DMH+EESG	11.66±2.10[b]	10.66±0.42[b]	1.66±0.16[c]
Distal colon			
Control	0	0	0
DMH	32.5±2.5	19.5±0.99	3.16±0.47
DMH+5-FU	17.5±2.14[a]	13.33±1.08[a]	2.83±0.30
DMH+EESG	19.16±3.00[b]	11.66±0.84[a]	1.5±0.34[b]
Total colon			
Control	0	0	0
DMH	35±4.28	25±3.65	3.5±0.5
DMH+5-FU	24.16±2.17[c]	17.83±2.94	1.83±0.3[b]
DMH-+EESG	20.83±2.38[b]	13.16±1.53[b]	2.16±0.3[b]

Values are mean ± SEM, n=6 in each group, statistically significant b**p<0.01 c*P<0.05 a***P<0.001 when compared with disease control (DMH treated) group (One way ANOVA fallowed by Dunnet's test).
EESG=Ethanolic Extract of *Sesbania grandiflora*; DMH=1, 2 Dimethyl hydrazine.

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

Group/ Treatment	Number of aberrant crypts per ACF				
	1	2	3	4	≥5
Proximal Colon					
Control	0	0	0	0	0
DMH	8.33±0.61	5.5±0.88	5.33±0.71	4.5±0.71	3.66±0.55
DMH+5-FU	4.66±0.88[b]	4.16±0.83	2.83±0.79[c]	2.33±0.42[c]	1.83±0.54[c]
DMH+EEAM	4.33±0.88[b]	3±0.73	2.5±0.61[c]	2±0.51[b]	1.5±0.34[b]
Distal colon					
Control	0	0	0	0	0
DMH	18.33±1.08	17.33±0.71	16.83±1.66	13.5±0.67	11.66±0.66
DMH+5-FU	14.5±1.58[c]	14.33±0.84[c]	16±1	11.16±0.54[c]	9.33±0.66[c]
DMH+EEAM	12.16±0.54[b]	13.66±0.8[b]	11.16±0.83[b]	10.83±0.4[b]	8.33±0.42[a]
Total colon					
Control	0	0	0	0	0
DMH	24.5±0.42	22.83±0.6	22±0.68	20.83±0.98	20±1.03
DMH+5-FU	18.83±1.01	18.33±1.28[b]	16.16±0.74[b]	17.5±0.84[c]	16.5±0.84[c]
DMH+EEAM	15±0.81	19.83±1.01	13.33±1.08[a]	14.66±0.98[b]	14±1.12[b]

Values are mean±SEM, n=6 in each group, statistically significant [a]***p<0.001, [b]**p<0.01, [c]*p<0.05when compared with disease control (DMH treated) group (One way ANOVA fallowed by Dunnet's test). EESG=Ethanolic extract of *Sesbaina grandiflora*; DMH=1, 2 Dimethyl hydrazine

Table 4: Effect of Prophylactic treatment of ethanolic extract of *Sesbaina grandiflora* and DMH induced colon cancer apoptotic index

Group/treatment	Apoptotic index
Control	2.11±0.16
DMH	0.85±0.09
DMH+5-FU	0.95±0.13
DMH+EESG	1.38±0.16[c]

Values are mean ± SD, n=6 in each group, statistically significant c*p<0.05. When compared with disease control (DMH treated) group (One way ANOVA fallowed by Dunnet's test). EESG=Ethanolic Extract of *Sesbania grandiflora*; DMH=1,2 Dimethyl hydrazine.

Table 5: Effect of ethanolic extract of *Sesbaina grandiflora* on various haematological parameters on 28th day

Haematological parameters	Control	DMH	DMH+5-FU	DMH+EESG
RBC(cells/µL×10^3)	7.66±0.55	8.1±0.98	14.33±0.66[b]	15.16±1.74[a]
WBC(cells/µL×10^3)	10.33±0.61	8.93±0.26	11.33±0.95[c]	11.16±0.40[c]
HGB(g/dL)	18.16±1.64	10.66±0.67	12.7±0.68	15.00±1.00[a]
PLT(cells/µL×10^3)	17.66±2.15	20±0.68	19±1.59	12.83±1.01[b]

Values are mean ± SEM, n=6 in each group, statistically significant b**p<0.01 a***p<0.001 c*p<0.05 when compared with disease control (DMH treated) group (One way ANOVA fallowed by Dunnet's test). EESG=Ethanolic Extract of *Sesbania grandiflora*; DMH=1,2 Dimethyl hydrazine.

Table 6 : Effect of ethanolic extract of *Sesbaina grandiflora* on relative weight of organs in rats treated with DMH.

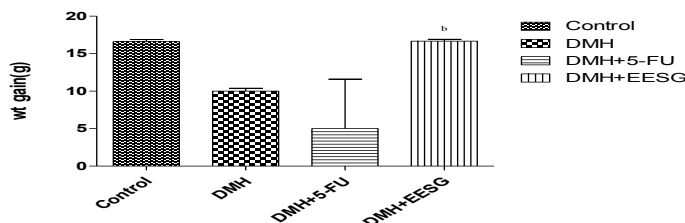| Relative Wt. of organs(Wt. of organ/100g)	Control	DMH	DMH+5-FU	DMH+EESG
Liver	7.14±0.02	8.36±0.22	8.61±0.09	7.68±0.18[c]
Kidney	0.82±0.00	0.81±0.01	1.38±0.08[b]	0.84±0.00[a]
Heart	0.59±0.07	0.73±0.04	0.55±0.02[c]	0.59±0.04
Lungs	1.37±0.04	1.68±0.04	1.51±0.07	1.36±0.04[a]
Pancreas	1.25±0.12	1.46±0.10	1.52±0.09	1.31±0.05[b]
Spleen	0.73±0.00	0.75±0.01	0.76±0.00	0.73±0.01
Colon	1.41±0.04	1.3±0.05	1.36±0.04	1.35±0.04
Stomach	1.18±0.01	1.34±0.01	1.31±0.02[a]	1.27±0.02[a]

Values are mean ± SEM, 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 fallowed by Dunnet's test). EESG=Ethanolic Extract of *Sesbania grandiflora*; DMH=1, 2 Dimethyl hydrazine.

IN VITRO METHODS

Table 7: Effect of ethanolic extract of *Sesbania grandiflora* on lipid peroxidation:

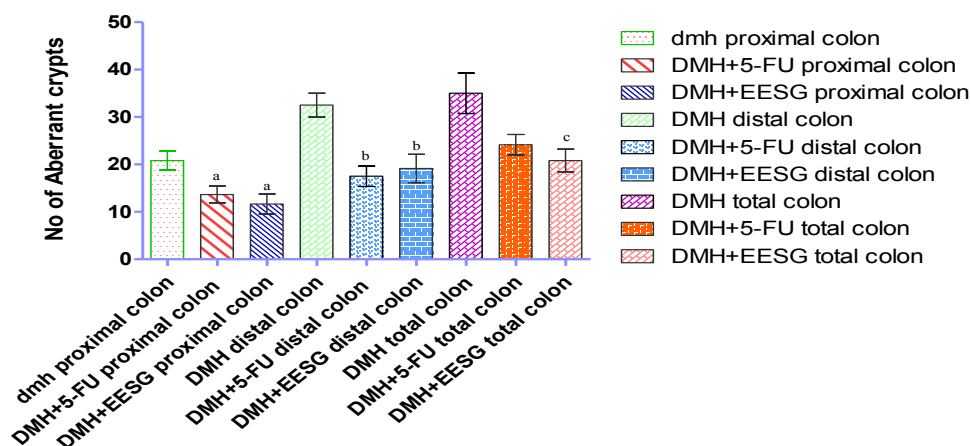| Group/Treatment	Nmol/ml
Control	0.22±0.01
DMH	0.65±0.007
DMH+5-FU	0.45±0.009[a]
DMH+EESG	0.24±0.019[a]

Values are mean±SEM, n=6 in each group, statistically significant a***P<0.001. When compared with disease control (DMH treated) group (One way ANOVA fallowed by Dunnet's test). EESG=Ethanolic Extract of *Sesbania grandiflora*; DMH=1, 2 Dimethyl hydrazine.

Table 8: Measurement of reduced GSH

Group	Mg/g
Control	0.35±0.009
DMH	0.22±0.007
DMH+5-FU	0.43±0.004[b]
DMH+EESG	0.37±0.01[a]

Values are mean±SEM, 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 fallowed by Dunnet's test). EESG=Ethanolic Extract of *Sesbania grandiflora*; DMH=1,2 Dimethyl hydrazine.

Table 9: Estimation of ascorbic acid in rats treated with DMH and EESG

Group	Umg/protein
Control	4.53±0.008
DMH	1.92±0.004
DMH+5-FU	0.53±0.004[a]
DMH+EESG	4.43±0.008[a]

Values are mean±SEM, n=6 in each group, statistically significant a***P<0.001 when compared with disease control (DMH treated) group (One way ANOVA fallowed by Dunnet's test). EESG=Ethanolic Extract of *Sesbania grandiflora*; DMH=1,2 Dimethyl hydrazine.

Fig 1: Body weight changes on treatment with ethanolic extract of *Sesbania grandiflora* in DMH induced colon cancer rats on 28th day.

Values are mean ± SEM, n=6 in each group, statistically significant [b]**P<0.01 when compared with disease control (DMH treated) group (One way ANOVA fallowed by Dunnet's test). EESG=Ethanolic Extract of *Sesbania grandiflora*; DMH=1, 2 Dimethyl hydrazine
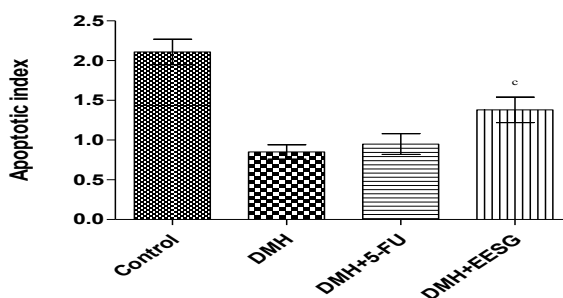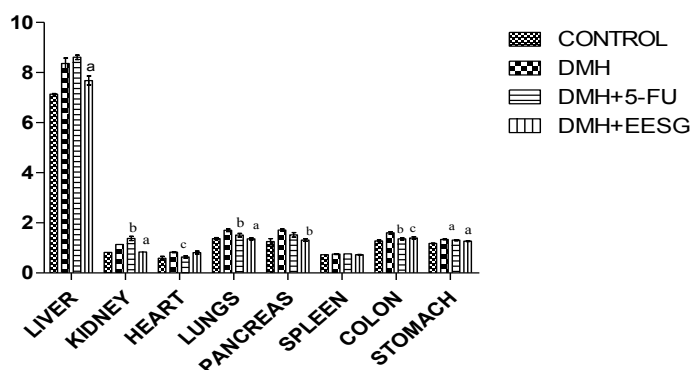
Fig 2: Regional distribution of aberrant crypt categories (1, 2, 3, 4 and ≥ 5) in rats treated with DMH and EEAM.

Values are mean±SEM, n=6 in each group, statistically significant a***$p<0.001$, b**$p<0.01$, c*$p<0.05$ when compared with disease control (DMH treated) group (One way ANOVA fallowed by Dunnet's test). EEAM=Ethanolic extract of *Sesbania grandiflora*; DMH=1, 2 Dimethyl hydrazine.

3. Apoptosis index:

Fig 3: Effect of Prophylactic treatment of ethanolic extract of *Sesbaina grandiflora* and DMH induced colon cancer apoptotic index

Values are mean ± SEM, n=6 in each group, statistically significant c*$p<0.05$. When compared with disease control (DMH treated) group (One way ANOVA fallowed by Dunnet's test). EESG=Ethanolic Extract of *Sesbania grandiflora*; DMH=1,2 Dimethyl hydrazine.
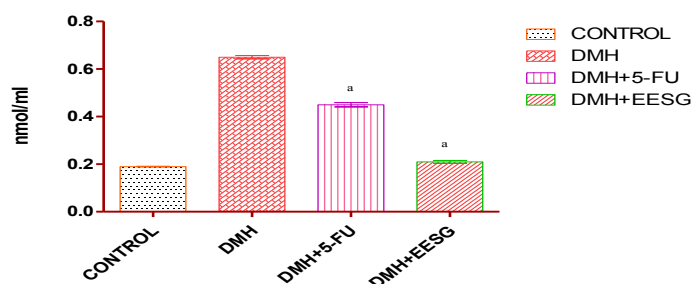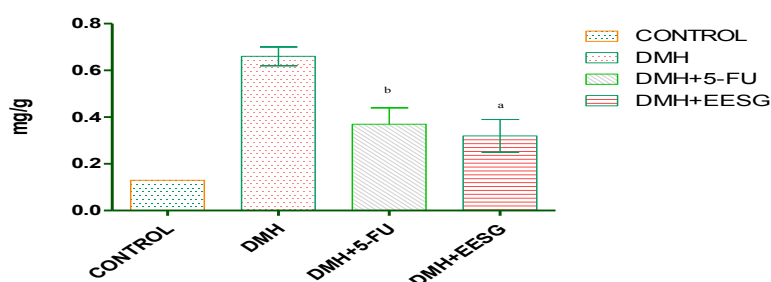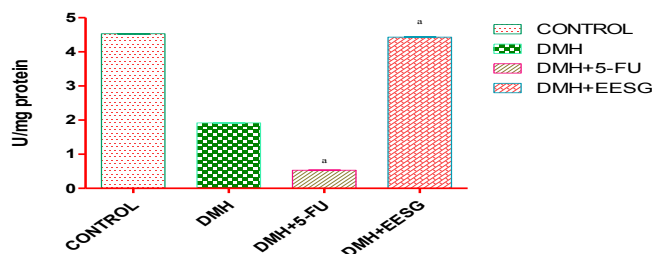
Fig 5: Effect of ethanolic extract of *Sesbaina grandiflora* on relative weight of organs

Values are mean ± SEM, n=6 in each group, statistically significant c*P<0.05 b**P<0.01 a***P<0.001 when compared with disease control (DMH treated) group (One way ANOVA fallowed by Dunnet's test). EESG=Ethanolic Extract of *Sesbania grandiflora*; DMH=1,2 Dimethyl hydrazine.

INVITRO METHODS

Fig 6: Measurement of Lipid Peroxidation

Values are Mean±SEM, n=6 in each group, statistically significant a***P<0.001 when compared with disease control (DMH treated) group (One way ANOVA fallowed by Dunnet's test). EESG=Ethanolic Extract of *Sesbania grandiflora*; DMH=1,2 Dimethyl hydrazine.

Fig 7: Measurement of reduced GSH

Values are Mean±SEM, 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 fallowed by Dunnet's test). EESG=Ethanolic Extract of *Sesbania grandiflora*; DMH=1,2 Dimethyl hydrazine.

Fig 8: Estimation of ascorbic acid in rats treated with DMH and EESG

Values are mean ± SEM, n=6 in each group, statistically significant a***P<0.001 when compared with disease control (DMH treated) group (One way ANOVA fallowed by Dunnet's test). EESG=Ethanolic Extract of *Sesbania grandiflora*; DMH=1,2 Dimethyl hydrazine.

Fig 9: Colon crypt pathology

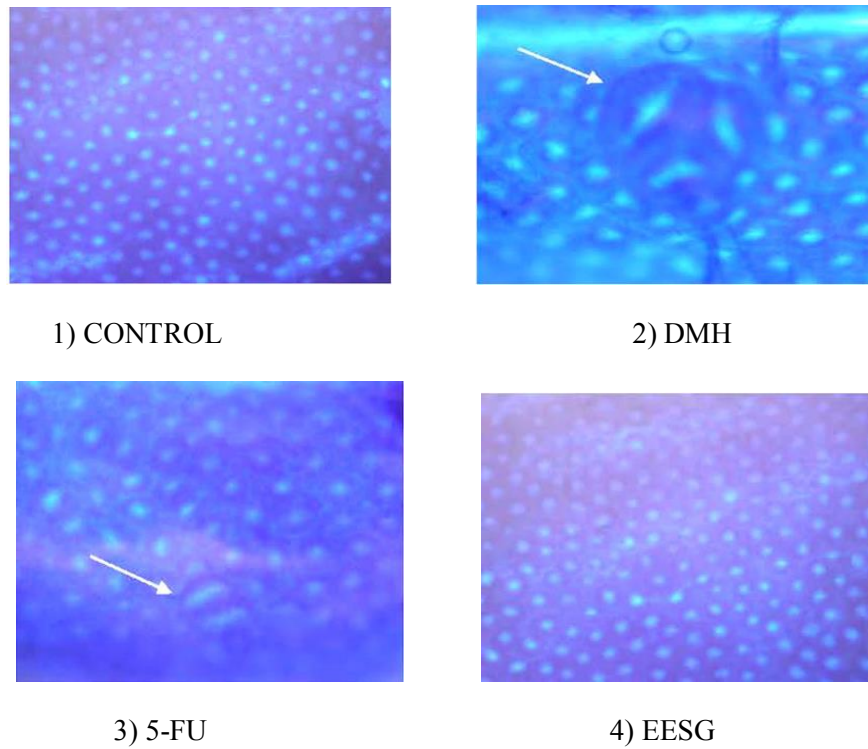1) CONTROL

2) DMH

3) 5-FU

4) EESG

Fig 9: Topographical view of normal crypts and ACF (arrows) in the colonic mucosa stained with Methylene blue 1) normal crypts (40x). 2) DMH treated showed three aberrant crypts (40x). 3) 5-FU treated showed single crypt (40 xs). 4) EESG showed normal crypts (40 xs).

Fig 10: *Histopathology*

A)   CONTROL

B) DMH Treated
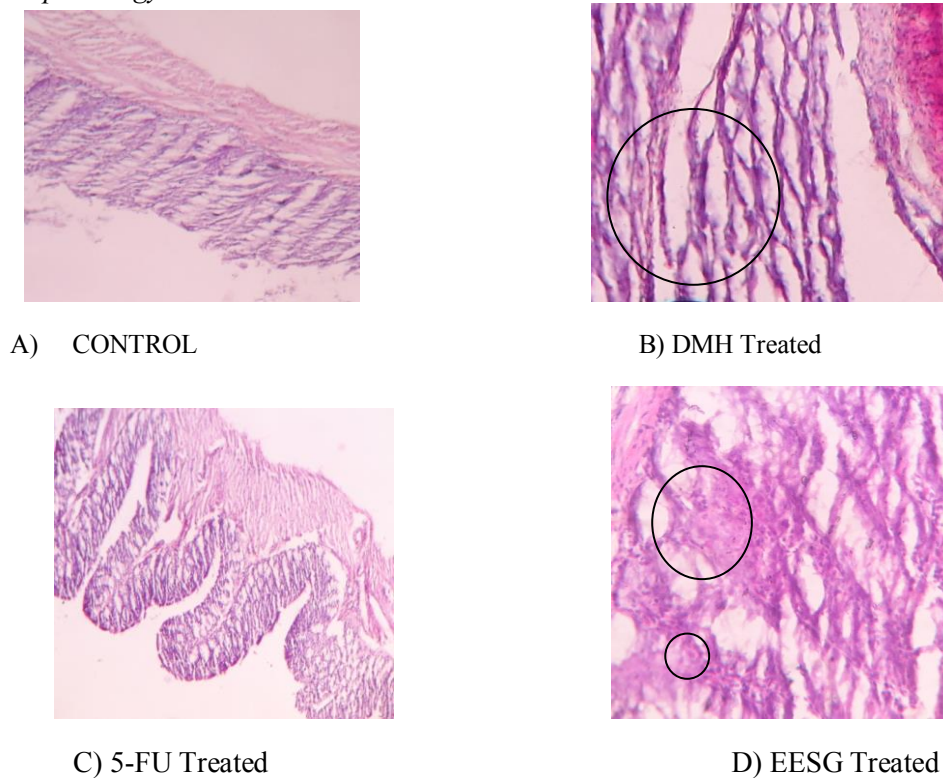

C) 5-FU Treated

D) EESG Treated

Fig 10: A) H&E stained section shows mostly stroma composed of mucosa lined by single layer of epithelial cells with basal placed nucleus & large amount of esnophilic cytoplasm (40x). B) Mucosal glands lined by multi layered epithelial cells. Glands showing nucleus overcrowding with hyperchromatic. ACF Count 8/10 high power field (HPF) (40x). C) Formation of polyps but no malignance. D) Mucosal gland is lined by single layered epithelial cells with basal placed nucleus and large amount of clear to esnophilic cytoplasm. Some areas showing ACF formation 2/10 HPF with occasional inflammatory infiltrates (40x).

Whereas in treatment with EESG pods at dose of 400mg/kg body weight as shown (Table 1) increased significantly the weight gain. The growth features of ACF and dysplastic ACF and their location as measure of the biological efficacy of the modifier of colon carcinogenesis (Bird *et al.,* 1995). ACF and dysplastic ACF may represent the earliest detectable lesion in the development of colon cancer. Various studies also suggest that dysplastic ACF/ACF are precursor of colon cancer in human and rodents (Mori *et al.,* 2005). In our present study increased crypt size was observed in DMH-treated rats and more over at the end of 2 weeks study the percentage of ACF in rats treated with DMH alone was 90% and each continued not less than four crypts/foci. On the hand, when the chemopreventive efficacy of EESG was investigated against DMH-induced putative preneoplastic foci in rat colon, it became clear that this colorant reduced the number of aberrant crypts/rat, but not the induction of ACF itself, when it was administered after carcinogen treatment. We also observed that the inhibitory effect of EESG on development of ACF and dysplastic ACF was pronounced in the entire period treatment regimen as compared to treatment group. Reported that no ACF were found in the proximal colon after DMH, at a site where carcinomas eventually occur; they concluded that the carcinomas that form in the proximal colon seldom if ever arise from an ACF, and that consequently, the location, number, and size of the ACF that occur early after DMH exposure do not correlate with the location or predict the incidence of carcinomas that eventually form in the colon. Based on the results it also showed that EESG induces apoptosis the apoptotic index was more in rats treated with DMH and EESG than rats treated with DMH alone. Colorectal epithelial homeostasis is dependent not only on the rate of cell production but also on apoptosis, agenetically programmed process of autonomous cell death. Inhibition of apoptosis is an integral component of the genesis of colorectal adenomas and carcinomas. The inhibition of apoptosis by bcl-2 in follicular lymphomas in chronic myeloid leukemia is known to provide a selective growth advantage (Bedi 1994) as well as confer resistance to anticancer agents (Fisher *et al.,* 1994). Aberrant cell survival could also promote the accumulation of secondary genetic changes that lead to neoplastic progression. Therefore, the inhibition of apoptosis in colorectal neoplasms may contribute to tumor growth, clonal evolution, and inherent resistance to chemotherapeutic agents. Treatment with EESG pods at a dose of 400mg/kg body weight as shown (Table 5) increased significantly the haemoglobin content, RBC count, WBC content and platelets increased significantly and decreased in platelets in DMH group as compared to control group. Treatment with EESG restored the haematological parameters to more or less control values. The number of RBC, WBC count, haemoglobin content also decreased significantly when compared to that of DMH group. In cancer chemotherapy the major problems are myelosuppression and anaemia (Maseki *et al.,* 1981). The anaemia encountered in tumour bearing rats is mainly due to reduction in RBC,WBC and haemoglobin percentage and this may be due to iron deficiency or due to haemolytic or myelopathy conditions (Fenninger and Mider 1954). Similar results were observed in (Table 5) the present study in animals of the DMH group. The reversal of haematological parameters indicates that the extract posses protective action on the hematopoietic system. This reinstates that use of herbs might be more effective strategy in the treatment of cancer. Reduced circulatory lipid peroxidation associated with antioxidant depletion was observed in DMH-induced colon bearing animals in table 7. Malondialdehyde is final product of oxidative stress and is good indicator for extent of oxidative stress (Sekizuka 1988). Myeloperoxidase catalyses

the conversion of proportionally more stable hydrogen peroxide to unstable hydrochlorus acid. This in turn promotes oxidative stress and additionally it induces neutrophil infiltration on mucosal area causing further damage to the tissue (Halliwell 1995). Earlier reports suggest that tumor cells substantial amount of H_2O_2 that are released into circulation (Manju V and Nalini N 2005). In addition superoxide (O_2^-) and hydroxyl radicals (OH) are also released into circulation resulting in increased susceptibility of the plasma and RBC to lipid peroxidation in DMH-treated rats. EESG is known to possess antioxidant property and scavenge free radicals like superoxide and hydroxyl radicals (Pollard *et al.,* 2006). In our study we observed decreased levels of TBRS on EESG supplementation. Antioxidant enzymes such as CAT are widely distributed in all cells. The enzymes help to protect against O_2^- and H_2O_2 mediated lipid peroxidation. In table 9 the observed increase in circulating lipid peroxides of DMH-treated animals in the present study correlate with the decline in circulatory antioxidants CATS. This may be due to their over utilization to scavenge the products of lipid peroxidation.

ACKNOWLEDGEMENTS

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