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RESEARCH ARTICLE

HEPATOPROTECTIVE ROLE OF *BASELLA RUBRA* ON 1, 2-DIMETHYLHYDRAZINE INDUCED COLON CARCINOGENESIS IN RAT MODEL

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Abstract

The main objective of the study is to determine the hepatoprotective efficacy of *Basella rubra* against chemically induced colon carcinogenesis model. The study was performed in male Wistar rats allocated into six groups. First group considered to be normal control and second group as drug control receives *Basella rubra* aqueous extract (BRAE 250mg/kg b.w.) orally for 30 weeks, third group served as cancer control injected with only DMH (25mg/kg b.w.) for 15 consecutive weeks. Rats in group IV and VI were administered with BRAE along with DMH injections. Rats in group V were administered with BRAE after cessation of DMH injection. After 30 weeks of experimental period, all the organs were collected and the liver tissue from all groups of rats was subjected to lipid peroxidation (LPO) and histological studies. Protective effects of BRAE were observed by increased in consumption of food and body weights in BRAE administration groups. Increased levels of LPO were seen in only DMH injected rats; BRAE administration to DMH injected rats significantly decreased the levels of LPO in liver tissue. Histopathological findings revealed that high level of damage was seen in only DMH induced rats whereas administration of BRAE shows recovery in liver architecture and the improved results were observed in entire period administration of BRAE. From the above results, it can be concluded that *Basella rubra* possess the hepatoprotective effect against chemically induced colon carcinogenesis in rats. The effect was more pronounced in entire period administration of BRAE.

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INTRODUCTION

Colorectal cancer is the third common form of all diagnosed cancers, found in both men and women. An estimated of 93,090 cases of colon cancer are to be diagnosed in 2015 (American cancer society, 2015). Numerous reports stated that, close association between the diet and incidence of colon cancer (Katia et al. 2013). It has been estimated that, modifications in the dietary habits prevent 70-80% of colorectal cancer (Denis et al. 2002). Diet is the most imperative factor in regulating colon cancer development. Therefore extensive research has been made to produce dietary chemopreventive agents that could inhibit, delay or reverse the multistage carcinogenesis (Eun et al. 2007).

1, 2-dimethylhydrazine (DMH) broadly used as an experimental model to study colorectal carcinogenesis in rodents. The establishment of the first animal model was appreciated for its macroscopic and histological similarity to human colorectal carcinoma (Martina et al. 2005). DMH is initially metabolized in liver to form azoxymethane and methylazoxymethane which is then transported to colon via bile or blood to produce ultimate

carcinogenic metabolite, diazonium ion that elicits an oxidative stress by methylating biomolecules of colonic epithelial cells and that leads to promutagenic events leads to inflammation and tumor promotion (Hamiza et al. 2012). 1, 2-Dimethyl hydrazine (DMH) is known to generate hydroxyl radical in the existence of metal ions that assist to the initiation of lipid peroxidation. In DMH-induced animal model circulation of increased levels or progressed generation of ROS and toxic degradation products of lipid peroxidation have been reported. Free radicals or reactive oxygen species (ROS) are the main offenders in lipid peroxidation and forms destructive and irreversible damage to the components of a cell, such as lipids, proteins and DNA (Bouftira et al. 2012).

Generally normal levels of ROS are capable of participated in signal transduction and intercellular communications. Excess production of these reactive oxygen species cause various pathological effects such as causing lipid peroxidation, protein peroxidation, DNA damage and cellular degeneration leading to cardiovascular disease, inflammatory disease, cancer and other disorders. To maintain homeostatic balance between the free radicals production and our defense system of the body, there is require of diet rich with antioxidants (Manish et al., 2011).

Antioxidants are the compounds that possess the property of inhibiting the initiation or propagation of oxidizing chain reaction. Natural antioxidants refer to compounds that are derived from plant sources. Based on the type and source of the material used, the mode of action of natural antioxidants involves multiple actions. It is believed that the regular consumption of dietary antioxidants may reduce the risk of several serious diseases (Tawheed et al. 2014). Consumption of natural antioxidants in fruits and vegetables, favors the xenobiotic metabolizing enzymes to act against cardiovascular disease and cancers. Hence, identification of dietary constituents that prevent colon cancer is important and a major focus of research in recent years. Recently, there has been a keen interest in the protective and therapeutic effects of certain plant chemicals on chronic diseases including cancer. Especially, active compounds in dietary phytochemicals that consist of a wide variety of biological applications have drawn a great deal of attention from both the scientific community and the general public owing to their demonstrated ability to suppress cancers (Balaiya et al. 2010). Therefore, prevention of cancer through diet is regard as safety and economic, compare synthetic anticancer drugs with prolong survival and minimal side effects.

Basella rubra belongs to family Basellaceae, consumed as a green leafy vegetable throughout the country and grown abundant in Asian counties. It has been cited in Indian traditional medicine for various applications, mainly in the treatment of ulcer, gonorrhea and balanitis (Nagarajan et al. 2012) and in Chinese traditional medicine, *B. rubra* has been used for the treatment of constipation, as a diuretic and as toxicide (Toshiyuki et al. 2001). *Basella rubra* has been reported to possess antioxidant (Nagarajan et al. 2012), anti-inflammatory (Bhanu priya et al. 2014), anti-diabetic (Nirmala et al. 2011), hepatoprotective (Yanadiah et al. 20011) and also anti-microbial (Sen et al. 2010) properties. Inspite of numerous therapeutic effects of *B. rubra*, it effects on its hepatoprotective role in chemically induced colon carcinogenesis model has not been reported yet. Since, the present investigation study examines the protective efficacy of BRAE on oxidative stress induced by DMH colon carcinogenesis. The effects were evaluated by the morphological observations, measurement TBRAS levels and by histoarchitecture determination.

2. MATERIALS AND METHODS

2.1 Plant collection and authentication

The *Basella rubra* leaves were collected from in and around the regions of Tirupati, Andhra Pradesh, India in the month of October to November 2012 depending on its easy availability. The plant materials were identified and authenticated (Herbarium. No. SPMVV/BT/BR1/2012) by Dr. N. Nagaraju, Assistant professor, Dept. of Botany, Sri Venkateswara Arts College, Tirupati, Andhra Pradesh.

2.2 Preparation of aqueous extraction

The leaves of *Basella rubra* was washed with tap water in order to remove any dust particles and then dried the leaves in shade for 2 weeks. The dried leaves were ground to fine powder using electric grinder. The 10g of powder was weighed out and mixed with 500 ml distilled water. This mixture was then stirred for 16 h. The supernatant mixture was then filtered and then freeze dried. The freeze dried material was weighed and re-dissolved in distilled water to produce a stock solution that was stored at frozen -20°C for further studies.

2.3 Animal maintenance

In this study Male Wistar albino rats were selected and procured from Ragavendra enterprises, Bangalore, India. All Rats were placed in polypropylene plastic cages covered with wire mesh top and a hygienic bed of husk in a specific-pathogen free animal room. Room temperature was maintained at $22\pm2^{o}C$ and a humidity of 55 ± 5 % under a 12h light-dark cycle. The animals were acclimatized to laboratory conditions at least one week before starting the experiment and they had free access to food and water *ad labitum*. The study protocol was performed as

per institute animal ethics committee regulations and approved by the committee (Reg. No. 1077/PO/a/12/CPCSEA/SPMVV-IEC/2014/02).

2.4 Carcinogen treatments

The experimental rats were divided into six groups. The rats in groups III, IV,V & VI received sub-cutaneous injections of DMH at a dose of 25 mg/kg body weight once a week for the fifteen consecutive weeks to induce colorectal cancer. Prior to subcutaneous injection, DMH was dissolved in 1mM EDTA (freshly prepared before use) the pH adjusted to 6.5 with 1mM NaOH to ensure the pH and stability of the chemical and was used immediately after preparation.

2.5 Experimental design

All the rats were randomly allocated into six groups; each group consists of six rats. Rats in group I received normal saline during the experimental period served as control. Group II rats received only BRAE (250mg/kg/day b.w.) through oral dosage for 30 weeks served as drug control. Group III rats served as carcinogen control received DMH injections (25 mg/kg b.w.) subcutaneously once a week for 15 weeks and then animals were kept without any treatment until 30 weeks. Group IV rats received DMH (same as Group III) and meanwhile BRAE (250mg/kg/day b.w.) administration were started one week before the starting of the experiment and continued up to final exposure of carcinogen (0-15 weeks). Group V rats received DMH (same as Group III) and also administration of BRAE (250mg/kg/day b.w.) started after the DMH termination and proceeds until the end of experiment (16-30 weeks). Group VI rats received DMH (same as Group III) and also BRAE was administered from day one of the experiment (1-30 weeks) and continued for entire period of the experiment.

2.6 Body weights, food consumption and Tissue pathology

The body weights of rats in all the groups were monitored throughout the study period and they were recorded once a week for 30 weeks. Food consumption of the rats in all the groups was also observed during the experimental period for once a week (Jun et al. 2002).

After necropsy, a subjective examination of organs (Spleen, liver, kidneys & colon) were collected from all the rats in different groups and they were observed for morphological changes and signs of toxicity. Organs were weighted and they were preserved for further study (Dias et al. 2006). The colons were removed and flushed clear with ice cold physiological saline (0.9% NaCl solution). Colons were opened longitudinally along the median and laid flat to examine the incidence of macroscopic lesions (Takefumi et al. 2006).

2.7 Lipid Peroxidation Assay

A weighed portion of liver from the rats in different groups was homogenized in phosphate buffer (100 mM, pH 7.0) and used to measure the concentration of thiobarbituric acid reacting substances (TBARS) as an indicator of lipid peroxidation. The extent of lipid peroxidation was measured in terms of formation of TBARS in liver tissue by the method of Ohkawa et al. (1979). 5% liver homogenate was prepared in ice cold 1.5% KCL. To 200µl of liver homogenate, 50µl of 8.1% sodium dodecyl sulphate and 375 µl of thiobarbituric acid (0.6%) TBA was added and the mixture was placed in boiling water bath for 60 min at 95^0C and then samples were allowed to cool at room temperature. A mixture of 1.25ml of butanol: pyridine (1.5:1) was added, vortexed and centrifuged at 1000rpm for 5 minutes. The pink colored chromogen was formed and it was produced by the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA), a secondary product of lipid peroxidation measured was estimated at 532 nm in UV-VIS spectrophotometer. The values were expressed as the nmol/ TBARS formed per gram tissue.

2.8 Histological examination

Liver tissues were cut in small pieces and immersed in neutral buffered formalin for 24 h. The fixed tissues were processed for embedded in paraffin, sectioned, deparaffinized and rehydrated using the standard techniques (Bancroft and Gamble, 2002). The extent of hepatic damage was evaluated by assessing the morphological changes in the liver sections of 5–6 µm were cut and stained with hematoxylin and eosin and examined for histopathological changes.

2.9 Statistical analysis

The values obtained from results were articulated as the mean $\pm$ standard deviation (SD). The statistical significance of the data was evaluated by One-way analysis of variance (ANOVA) followed by Dunnett's Comparision Test. The statistical significance was considered at $*p<0.05$.

3. RESULTS

3.1 General findings

Body weights were recorded weekly during the experimental period of 30 weeks. Various changes were observed in different group rats regarding their body weights. Body weights of the rats were shown in Table-1. Rats in all the groups gradually increased their body weights. In only BRAE administration (group II) rats body weights

were elevated as like control group rats (group I) for the period of the experiment. The body weight gain was significantly decreased in DMH alone (group III) injected rats compared to control group rats (group I). Administration of BRAE to DMH injected (group IV, V & VI) rats shows significant increase in their body weights compared to cancer control group rats (group III). Significant increase in body weights were seen in group VI. At the end of the study the final body weights of all the rats were 273± 14.3, 269±13.8, 215±17.4, 243±14.1, 257±15.9 and 266±16.6g respectively.

Along with body weights, food consumption (g/day/rat) of the rats was also monitored every week for the period of experiment (Table 1). Food intake in group II rats (31.2± 4.3) was almost similar to control group I rats (32.4 ± 3.0). Average intake of food in rats injected with only DMH (group III) was significantly reduced (21.0 ± 5.2) compared to control group rats (group I). Whereas BRAE administration to DMH injected rats showed increased food consumption (group IV (25.9 ± 2.8), V (27.8 ± 4.3) and VI (29.2 ± 3.7)). This shows oral administration of BRAE has no adverse effects as observed by the body weights and also food consumption of rats.

At the end of the experiment (after 30 weeks), rats from all the groups were euthanatized and the intra-abdominal organs like liver, kidney and spleen were observed for gross morphological changes and they were weighted (g/100 g body wt). In drug control rats (group II) no noticeable changes were found, weights of the liver, kidney and spleen weights were (13.1±0.7, 2.19±0.1, 1.17±0.15) almost similar to control group rats (13.5±0.4, 2.2±0.1, 1.24±0.13). In only DMH injected rats (group III) shows abnormal changes macroscopically in their liver by formation of cyst (Figure 1) and organ weights (11.9±0.8, 1.8±0.1, 0.9±0.17) were reduced compared to control (group I) rat organs. BRAE administration to DMH injected rats in groups IV (12.5±0.4, 1.9±0.04, 0.9±0.19) group V (12.9±0.68, 1.9±0.14, 1.2±0.14) & group VI (13.1±0.68, 2.0±0.2, 1.2±0.16) organ weights were not significantly differ among the groups. Organ weights were significantly increased compared to only DMH injected rats (group III).

3.2 Lipid Peroxidation (LPO)

The effect of BRAE on the levels of oxidative stress marker (TBARS) in the liver homogenate was given in Table 2. The level of lipid peroxidation was in the order of 0.30±0.03, 0.31±0.02, 0.54±0.04, 0.39±0.04, 0.35±0.02 and 0.34±0.02 nmoles of TBARS formed/g tissue/hr in control rats, drug control, only DMH, Initiation, post – initiation and entire period groups respectively. The level of TBARS in liver homogenate was significantly elevated in only DMH injected rats when compared to control (group I) and drug control rats (group II). BRAE administration to DMH induced rats ((group IV, V and VI) showed significantly ($p<0.05$) lower levels of TBARS. Restoration of TBARS to near normal levels was found in entire period administration of BRAE (group VI).

3.3 Histopathology

The histopathological changes in the liver sections are represented in figure 2. Liver histopathological examination was done after 30 weeks study to evaluate the histomorphological changes, the liver of control (group I) rats indicated the normal hepatic morphology with intact hepatocytes. Administration of only BRAE (group II) did not affect the hepatic architecture shows same normal hepatic morphology as like control rats, which shows protective nature of BRAE. The liver sections of only DMH injected rats (group III) showed fatty changes with vacuolation and scattered hepatocytes associated with necrosis. Administration of BRAE to DMH induced rats (group IV, V & VI) had displayed clear hepatic recovery by a complete regeneration of hepatocytes that are closely arranged and necrosis appeared to be normal with less vacuolation. The more pronounced recovery in hepatic morphology was seen in group VI rats (entire period administration of BRAE).

FIGURES

Table 1. Effect of BRAE on intake of food, body weights and organ weights of rats in different groups

General observations	Group I (Control)	Group II (Drug Control)	Group III (Only DMH)	Group IV (Initiation)	Group V (Post-initiation)	Group VI (Entire period)
Food consumption (g/rat/day)	32.4 ± 3.0	31.2± 4.3	21.0±5.2**	25.9 ± 2.8*	27.8±4.3	29.2±3.7
Initial body weight(g)	157± 11.2	155± 10.7	169±12.7	152±14.2	155±12.6	157±11.7
Final body weight (g)	273± 14.3	269±13.8	215±17.4**	243±14.1**	257±15.9*	266±16.6*

Organ weights (g)#						
Liver	13.5±0.4	13.1±0.7	11.9±0.8	12.5±0.4	12.9±0.68	13.1±0.68
Kidney	2.2±0.1	2.19±0.1	1.8±0.1	1.9±0.04	1.9±0.14	2.0±0.2
Spleen	1.24±0.13	1.17±0.15	0.9±0.17	0.9±0.19	1.2±0.14	1.2±0.16

All experimental values are expressed as mean ± SD. #Values represent after 30 weeks of experiment. Where ** $p<0.01$ and * $p<0.05$ compared with control group (Group I).

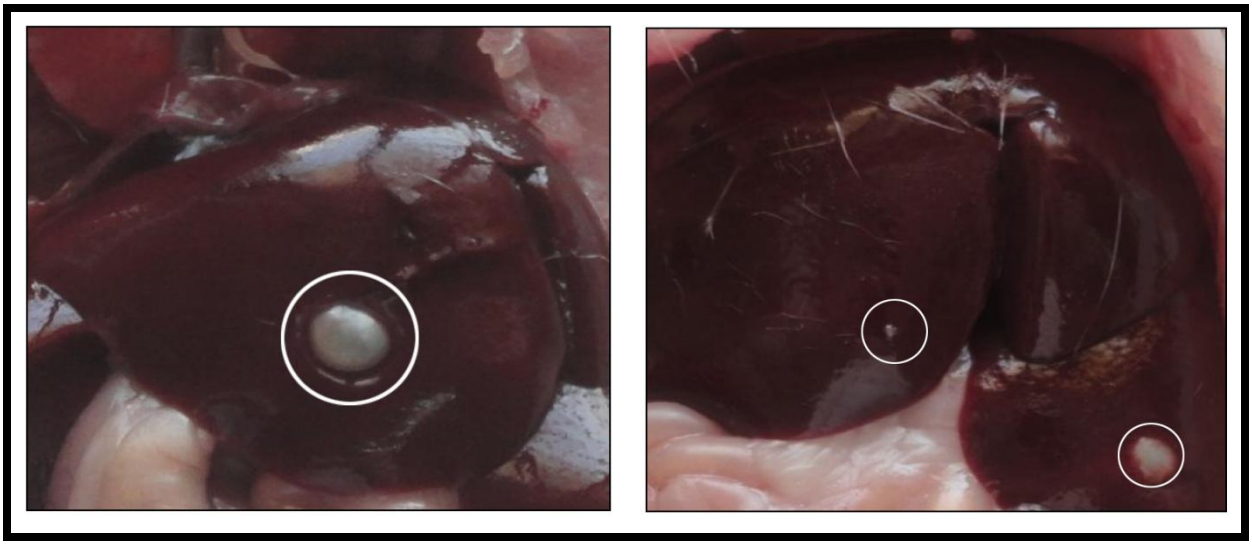

Fig. (1). Changes in the liver tissue. This figure shows the formation of cyst in the liver in only DMH induced rats, observed during euthanasia after 30 weeks of experiment.

Table 2. Effect of BRAE on liver TBARS levels

Groups	TBARS (nmol/g tissue)
Group I (Control)	0.30±0.03
Group II (Drug Control)	0.31±0.02
Group III (Only DMH)	0.54±0.04**
Group IV (Initiation)	0.39±0.04**
Group V (Post-initiation)	0.35±0.02*
Group VI (Entire period)	0.34±0.02

DMH- dimethylhydrazine; TBARS- thiobarbituric acid reactive substances. Experimental values are given as mean ± SD. Where ** $p<0.01$ and * $p<0.05$ compared with control group (Group I).

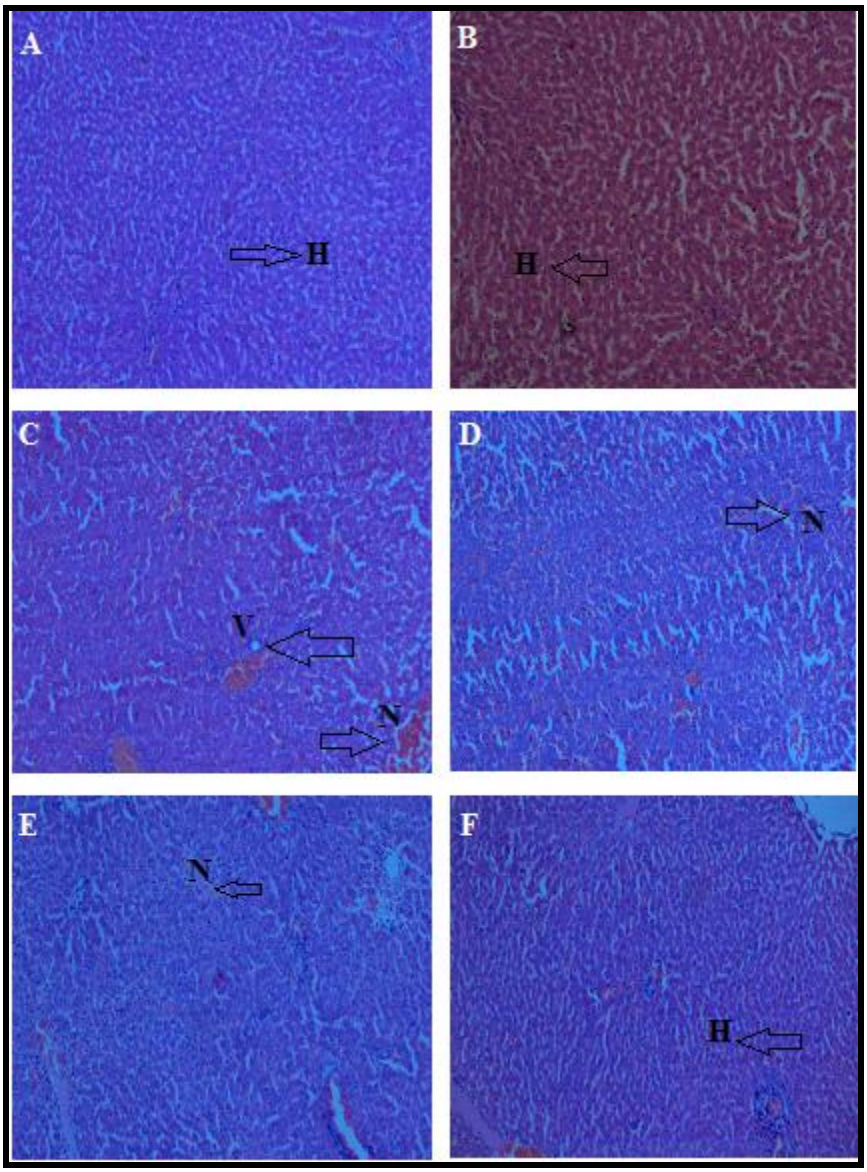

Fig. (2). Represents the liver H&E section (10 X Objective). A) Liver section from control rats with lack of vacuoles and intact hepatocytes. B) Section from drug control with no abnormalities. C) Section showing the liver from only DMH injected group shows the formation of vacuoles, scattered hepatocytes and necrosis. D) Liver section from initiation group with less vacuoles and necrosis. E) Section from post-initiation group with less necrosis and no vacuole formation. F) Liver section from entire period administration of BRAE shows the formation of intact hepatocytes and recovery from fatty changes. Arrow marks indicate H- Hepatocytes; V- Vacuoles; N- Necrosis.

4. DISCUSSION

Liver is more susceptible to injury because it is commonly exposed to agents (Environmental toxins, carcinogens and drugs) in their most reactive toxic forms. These agents cause liver damage by lipid peroxidation, oxidative damage, and free radical production. Even though free radicals are necessary to maintain biochemical processes and an aerobic metabolism but they are deleterious to health (Rojin et al. 2015). Oxidative stress is an imperative constituent of carcinogenesis. Free radicals produced as a result of oxidative stress initiate chain reactions that lead to the process of lipid peroxidation which causes injury to cell membranes. So agents that reduce free radical generation contribute to chemoprotection of cellular damage and also the carcinogenesis (Aditi et al. 2011).

Several reports explored that naturally occurring phenolics in fruits and vegetables as a combination can prevent cancer cell growth more efficiently than as a single compound (Halliwell, 2007). There are several studies reported that higher intake of fruits and vegetables have a protective role against colorectal cancer (Van et al. 2009). Everyone knows the importance of green leafy vegetables but their consumption was highly condensed and ignored. This study aims to highlight the significance of *Basella rubra* (leafy vegetable) in our regular diet. In the present study DMH (1, 2-dimethyl hydrazine) was selected to induce colon carcinogenesis in male Wistar rats. DMH shows the toxicity not only to target organ (colon) but also to non-target organs (liver) leads to metabolic alterations and occasionally malignant tumors of bile duct origin (Gilbert 1987). It is obvious that DMH could cause malignant tumors and pathological damage to the tissues of an animal. The cytoarchitectural and toxicological studies will give a clear understanding of the hepatoprotective effects of aqueous extract of *Basella rubra* against DMH induced colon carcinogenesis in rats.

Weight loss, decreased appetite and blood in the stool are frequently associated in colon cancer (Venkatachalam et al. 2013). Food intake was significantly decreased due to the increased occurrence of tumours in the colonic tract, which resulted in reduced weight in DMH exposed rats. In addition, enhanced development of tumour leads to growth rate inhibition. The general findings of our present study also showed reduction in body weight gain in only DMH injected rats. The intake of food also significantly decreased this may be due to the presence of tumors in the colonic tract. Oral administration of rats with BRAE in three different stages (groups) shows body weight restoration and the consumption of food also significantly increased. The results in present investigation reveal that the BRAE preventive potential role against DMH induced colon cancer.

DMH produces free radicals that induce oxidative DNA damage in the liver of rats (Ha-Na et al. 2007). Enhanced lipid peroxidation connected with depletion of antioxidant levels is a characteristic feature finding in cancer bearing rats which reflect excessive free radical production and excessive deployment of antioxidants (Manju and Nalini, 2007). The enhanced lipid peroxidation in the liver of DMH induced colon tumour bearing rats could be attributed to the DMH induced oxidative stress and production of reactive oxygen substances. Oxidative stress was measured by lipid peroxidation (LPO) levels in the liver homogenate. Numerous reports demonstrated the antioxidant potential of natural dietary agents and carotenoids that can significantly reduce free radicals and the oxidative load to help the body to healthy state (Aranganathan & Nalini, 2009; Sreedharan et al. 2009). The above reports were substantiated by our study as administration of BRAE to DMH injected rats (group IV, V and VI) significantly reduced hepatic TBARS levels and were restored to normal values (group I and II). However, hepatic TBARS levels were highly increased in only DMH injected rats (group III). This may be due to DMH, a methylating agent which releases oxidative stress and ROS. The antioxidant property of BRAE may be responsible for protecting the cells against the oxidative stress, possibly by increasing the endogenous defensive capacity of the liver to combat oxidative stress induced by DMH. Administration of BRAE to the entire period of the study was more effective than the other treatment stages. This effect of BRAE could be due to its strong antioxidant potential.

It is well well-known that almost all chemical carcinogens are metabolized in the liver (Heidelberger, 1977) and there is evidence that DMH is also metabolized in the same manner (Shank & Magee, 1967; Hawks & Magee, 1974). The evidence presented here indicates that rats treated with 25 mg/kg/wk b.w of DMH cause liver damage sufficient to impair normal hepatic function. Thus the rat liver metabolism of DMH may be changed during the course of an experiment in which DMH is administered by weekly injections (Castleden et al. 1979). Several studies reported that presence of atypical cells in the liver suggests that besides colonic tumors the liver may also be a site for abnormalities (Zhurkov et al. 1996), Jegatheeswaran et al. 2013 and Pamela et al. 2013). Gilbert (1987) reported that the dimethylhydrazine also has an effect on the liver, causing dysplasia, cyst formation, hamartomas and occasionally malignant tumors of bile duct origin. Our histopathological examination of liver sections in only DMH injected rats (group III) also revealed the hepatic damage and appearance of fatty changes with vacuolation and scattered hepatocytes associated with necrosis and results of this study were robustly agreed with above reports and also with the recent studies of Ansil et al. 2013 and Mitra et al. 2014. BRAE administered rats (group IV, V & VI) showed the signs of recovery from DMH induced hepatotoxicity and seems to prevent hepatic changes as evident from hepatic architectural pattern with mild to moderate depicted in figure-2.

BRAE markedly modulates the oxidative stress, thus *Basella rubra* may be an attractive candidate as an antioxidant supplement for anticancer therapy. Cumulatively, the study suggests that BRAE can protect liver tissues against DMH-induced colon carcinogenesis in rats. This protective and inhibitory effect of BRAE was more striking in the entire period administration of the study. The hepatopreventive activity exhibited by BRAE might be attributed to the presence of the single phytochemical constituents or in combination. This suggests that regular intake of *Basella rubra* in our diet may impart the hepatoprotective effects against harmful agents that leads to carcinogenesis.

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REFERENCES

Aditi Sharma, Krishan Kumar Sharma. (2011): Chemoprotective Role of Triphala Against 1, 2 Dimethylhydrazine Dihydrochloride Induced Carcinogenic Damage to Mouse Liver. Ind J. Clin. Biochem., 26(3):290–295.

American Cancer Society. (2015): Cancer Facts & Figures 2015. Atlanta.

Ansil, P.N., Jazaira, V., Prabha, S.P., Nitha, A., Latha, M.S. (2013): *Amorphophallus campanulatus* (roxb.) blume. tuber ameliorates hepatic oxidative stress during colon carcinogenesis induced by 1, 2 dimethylhydrazine. Int. J. Pharm. Pharm. Sci., 5 (1): 366-371.

Aranganathan, S., Nalini, N. (2009): Efficacy of the potential chemopreventive agent, hesperetin (citrus flavanone), on 1, 2-dimethylhydrazine induced colon carcinogenesis. Food Chem. Toxicol., 47:2594–2600.

Balaiya Velmurugan, Rana, P., Singh, Nidhi Kaul, Rajesh Agarwal, Chapla Agarwal. (2010): Dietary feeding of grape seed extract prevents intestinal tumorigenesis in APC $^{min/+}$mice. Neoplasia., 12 (1): 95-102.

Bancroft, J.D., Gamble, M. (2002): Theory and Practice of Histological Techniques, 5th ed. Churchill Livingstone, London, New York and Philadelphia.

Bhanu Priya, K., Venkata Subbaiah Kotakadi, Josthna, P. (2014): Anti-inflammatory Effect of *Basella rubra* on Oxazolone-induced Colitis in Rat. AJPCT., 2 (7): 832-841.

Bouftira Ibtissem, Hizem Hela, Mahmoud Amor,Chedlly Abdelly and Sfar Souad.(2012): Effect of *Mesembryanthemum crystallinum* Extract against DMH-Induced Colon Carcinogenesis in Experimental Animals. IJRPBS., 3 (3): 1038-1043.

Castleden, W.M., Shilkin, K.B. (1979): Diet, liver function and dimethylhydrazine-induced gastrointestinal tumours in male Wistar rats. Br. J. Cancer., 39: 731-739.

Denis, E., Corpet, Sylviane Tache. (2002): Most effective colon cancer chemopreventive agents in rats: A systematic review of Aberrant crypt foci and tumor data ranked by potency. Nutr. Cancer., 43 (1): 1-21.

Dias, M.C., Spinardi-Barbisan, A.L.T., Rodrigues, M.A.M., de Camargo, J.L.V., Tera, E., Barbisan, L.F. (2006): Lack of chemopreventive effects of ginger on colon carcinogenesis induced by 1,2-dimethylhydrazine in rats. Food Chem. Toxicol., 44: 877–884.

Eun Ji Kim, So Young Park, Hyun-Kyung Shin, Dae Young Kwon, Young-Joon Surh, and Jung Han Yoon Park. (2007): Activation of Caspase-8 Contributes to 3,3#-Diindolylmethane-Induced Apoptosis in Colon Cancer Cells. J. Nutr., 137: 31–36.

Gilbert, J.M. (1987): Experimental colorectal cancer as a model of human disease. Ann. R. Coll. Surg Engl., 69: 48-53.

Halliwell B. (2007): Dietary polyphenols: good, bad, or indifferent for your health? Cardiovasc Res., 73:341–347.

Hamiza, O.O., Muneeb, U., Rehman, Mir Tahir, Rehan Khan, Khan, A.Q., Lateef, A., Farrah Ali, Sarwat Sultana. (2012): Amelioration of 1, 2-dimethylhydrazine (DMH) induced colon oxidative stress, inflammation and tumor promotion response by tannic acid in Wistar rats. Asian Pac J Cancer Prev., 13: 4393-4402.

Ha-Na No, Hoonjeong Kwon, You-Gyoung Park, Choong-Ill Cheon, Jong-Sug Park, Taesun Park, Okezie I. Aruoma, Mi-Kyung Sung. (2007): Dietary quercetin inhibits 1, 2-dimethylhydrazine–induced liver DNA damage without altering colon DNA damage or precancerous lesion formation in rats. Nutrition Research., 27: 659–664.

Hawks, A., Magee, P.N. (1974): The alkylation of nucleic acids of rat and mouse in vivo by the carcinogen 1, 2-dimethylhydrazine. Br. J. Cancer., 30 (5): 440-447.

Heidelberger C. (1977): Chemical carcinogenesis. Cancer., 40: 430-433.

Jegatheeswaran, S., Mason, J.M., Hancock, H.C., Siriwardena, A.K. (2013): The liver-first approach to the management of colorectal cancer with synchronous hepatic metastases: a systematic review. JAMA Surg., 148: 385-391.

Jun Shimizu, Keita Kudoh, Masahiro Wada, Toshichika Takita, Satoshi Innami, Akio Maekawa, Tadahiro Tadokoro. (2002): Dietary curdlan suppresses dimethylhydrazine-induced aberrant crypt foci formation in Sprague-Dawley rat. Nutr. Res., 22: 867–877.

Katia Lofano, Mariabeatrice Principi, Maria Principia Scavo, Maria Pricci, Enzo Ierardi, Alfredo Di Leo. (2013): Dietary Lifestyle and Colorectal Cancer Onset, Recurrence, and Survival: Myth or Reality? J. Gastrointest. Canc., 44: 1–11.

Manish Kumar, Madhu Chandel, Subodh Kumar, Satwinderjeet Kaur. (2011): Protective effects of *Koelreuteria paniculata* Laxm. on oxidative stress and hydrogen peroxide-induced DNA damage. Phytopharmacology., 1(5):177-189.

Manju, V., Nalini, N. (2007): Protective role of luteolin in 1, 2-dimethylhydrazine induced experimental colon carcinogenesis. Cell Biochem. Funct., 25:189-94.

Martina Perse, Anton Cerar. (2005): The dimethylhydrazine induced colorectal tumours in rat - experimental colorectal carcinogenesis. Radiol. Oncol., 39(1): 61-70.

Mitra Dadrass, Vahid Nejati, Amir Tukmechi, Rahim Hobbenaghi. (2014): The Protective Effect of Cell Wall and Cytoplasmic Fraction of Selenium Enriched Yeast on 1, 2-Dimethylhydrazine-induced Damage in Liver. Res. Mol. Med., 2 (1): 38.

Nagarajan Anusuya, Rajkumar Gomathi, Sellamuthu Manian, Venkataramegowda Sivaram, Anita menon. (2012): Evaluation of *Basella rubra* L., *Rumex Nepalensis* Spreng. and *Commelina benghalensis* L. for antioxidant activity. Int. J. Pharm Pharm Sci., 4 (3): 714-720.

Nirmala, A.S., Saroja, G., Gayathri Devi. (2011): Phytochemical Screening and Antihyperglycemic Activity of *Basella rubra*. Recent Res Sci Technol., 3(11): 80-83.

Ohkawa, H., Ohishi, N., Yagi, K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

Pamela Christudoss, Ratnasamy Selvakumar, Anna Benjamin Pulimood, Jude Joseph Fleming, George Mathew. (2013): Protective Role of Aspirin, Vitamin C, and Zinc and their Effects on Zinc Status in the DMH-Induced Colon Carcinoma Model. Asian Pac J Cancer Prev., 14 (8): 4627-4634.

Rojin, T.S., Sukanya Shetty, Rajendra Holla. (2015): Hepatoprotective effect of *Mussaenda erythrophylla* and *Aegle marmelos* in ethanol induced rat hepatotoxicity model. Int. J. Appl. Biol. Pharm., 6(3): 223-228.

Sen, K. A., Goel, S., Rawal, N., Mahajan, S., Baboo, S., Alok. (2010): Antimicrobial Activity of Basella rubra leaves. IJPSR., 1 (2): 88-91.

Shank, R.C., Magee, P.N. (1967): Similarities between the biochemical actions of cycasin and dimethylnitrosamine. Biochem. J., 105(2): 521-527.

Sreedharan, V., Venkatachalam, K.K., Namasivayam, N. (2009): Effect of morin on tissue lipid peroxidation and antioxidant status in 1, 2-dimethylhydrazine induced experimental colon carcinogenesis. Invest. New Drugs., 27: 21-30.

Takefumi Katsuki, Keiji Hirata, Hideki Ishikawa, Nariaki Matsuura, Shin-ichiro Sumi, Hideaki Itoh. (2006): Aged Garlic Extract Has Chemopreventative Effects On 1, 2-Dimethylhydrazine-Induced Colon Tumors in Rats. J. Nutr., 136: 847-851.

Tawheed Amin, Monika Thakur. (2014): A Comparative Study on Proximate Composition, Phytochemical Screening, Antioxidant and Antimicrobial Activities of *Linum usitatisimum* L. (flaxseeds). Int. J. Curr Microbiol. App Sci., 3(4): 465-481.

Toshiyuki Murakami, Kazuhiro Hirano, Masayuki Yoshikawa. (2001): Medicinal Foodstuffs. XXIII. Structures of New Oleanane-Type Triterpene Oligoglycosides, Basellasaponins A, B, C, and D, from the Fresh Aerial Parts of *Basella rubra* L. Chem. Pharm. Bull., 49:776-9.

Van Duijnhoven, F.J., Bueno-De-Mesquita, H.B., Ferrari, P., Jenab, M., Boshuizen, H.C., Ros. (2009): Fruit, vegetables and colorectal cancer risk: the European Prospective Investigation into Cancer and Nutrition. Am. J. Clin. Nutr., 89:1441-1452.

Venkatachalam, K., Gunasekaran, S., Jesudoss, V.A., Namasivayam, N. (2013): The effect of rosamarinic acid on 1, 2-dimethylhydrazine induced colon carcinogenesis. Exp. Toxicol. Pathol., 65: 409-18.

Yanadaiah, J.P., Mohana Lakshmi, S., Jayaveera, K.N., Sudhakar, Y. (2011): Hepatoprotective activity of aqueous ethanolic extract of aerial parts of *Basella rubra* Linn against carbon tetrachloride and paracetamol - induced hepatotoxicity in rats. Int. J. Pharm Pharm Sci., 3(5): 502-506.

Zhurkov, V.S., Sycheva, L.P., Salamatova, O. (1996): Selective inhibition of micronuclei in the rat/mouse colon and liver by 1, 2-dimethylhydrazine – a seven tissue comparative study. Mut. Res., 12: 115-120.