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Chemo Preventive Dose of Wheatgrass in Experimentally Induced Colon Cancer in Rats

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1. Abstract

1.1. Introduction: Colon cancer is common gastrointestinal cancer. Role of wheatgrass as an alternative preventive therapy in colon cancer needs to be explored experimentally. Present study aimed at establishing minimum dose of wheatgrass with maximum protection for prevention of colon cancer in rats.

1.2. Materials and Methods: 36 male Sprague Dawley rats were split in 6 groups with each group having 6 animals. Weekly 30mg/kg body weight 1, 2- dimethyl hydrazine (DMH) was injected subcutaneously for 16 weeks to animals of cancer group. 6 animals in each group were given normal saline,120 mg alone wheatgrass and 80,100,120mg/kg body weight wheatgrass with DMH to 6 animals in each group. Biochemical and histological changes were done by standard methods.

1.3. Result: Lipid peroxidation (LPO) levels were significantly increased in those rats which were treated with DMH only (group 2) in comparison to control (group 1) while Glutathione (GSH) levels, catalase and Superoxide dismutase (SOD) were decreased significantly. LPO levels in DMH + wheatgrass groups were reduced with increasing dosage of wheatgrass from 80mg to 100mg and GSH, SOD and catalase decreased. 100mg and 120mg dosages of wheatgrass showed significant reduction in LPO and increase in GSH, SOD and catalase as compared to DMH group but not much difference between two groups.

1.4. Conclusion: This study concludes that minimum dose of wheatgrass providing maximum protection against colon cancer in rats is 100mg/kg body weight/day.

2. Introduction

Colorectal Cancers (CRC) are one among the foremost reason for mortality in western countries. It is estimated that worldwide 1.93 million new cases of CRC are diagnosed in 2020 [1]. Incidence of CRC differs in countries [2]. Rates of colorectal cancer (CRC) vary widely within Asia [2]. It is among the most common form of cancers in gastrointestinal tract and it is the fourth named after cancer post lung, prostate, and breast cancer [3].

Cancer of colon are linked to lifestyle and genetic alterations, therefore, without any intervention, a section of people are prone to have cancer of colon at any time during their lifetime [4]. Recent evidence prove that chronic state of hyperinsulinemia may cause the risk of colon cancer as excess intake of energy and some foreign foods e.g., saturated fats and refined carbohydrates induce insulin resistance and subsequent hyperinsulinemia [5]. Spectrum of aetiology of CRC vary from mutations in germline with genes of high penetrance like adenomatous polyposis coli (APC) genes to environmental factors and high body mass. Decades of evolving research have few results focussed on CRC primary prevention. In countries having high incidence reduced CRC mortality has been possible because of introduction of faecal occult blood test

(FOBT) screening, flexible sigmoidoscopy and colonoscopy [6].

In recent years, many experimental models of cancer have been applied to elucidate the molecular mechanisms involved in multi-stage process of carcinogenesis. To study colon cancer, carcinogen used is 1, 2-dimethylhydrazine (DMH). DMH induces tumours within the descending colon regardless of the mode of administration and histopathology coincides to that reviewed in human sporadic colon tumour [7]. DNA hydroxylated bases formation is a crucial event in chemical carcinogenesis [8].

Health and vitality both have been widely known to get boosted by cereal grasses like alfa-alfa, wheatgrass and barley grass both in humans and animals [9]. Wheatgrass is having abundant enzymes and chlorophyll. High (60%) chlorophyll is present in wheatgrass prevents the pathogen growth and is shown to inhibit the metabolic activity of carcinogen [10]. Thirteen vitamins, mostly having antioxidant property and other enzymes like cytochrome oxidase, superoxide dismutase (SOD) etc [11]., are present in wheatgrass. P4D1, which is known to stimulate renewal of RNA and DNA with an antioxidant effect is a glycoprotein present in wheatgrass. It is also thought to make cancer cells more prone to body's immune system. Despite of many people using wheatgrass as a dietary supplement, some supporters claim that wheatgrass diet, a common dietary program, can cause cancer regression, its progression can be influenced and can prolong lives of people with cancer too [12]. They trust that wheatgrass diet kills harmful bacteria in the digestive system, strengthens the immune system, and rids the body of toxins [13]. However, regarding its chemo preventive efficacy the information is still lacking. Detailed study to address the important changes in cell being influenced by wheatgrass need to be undertaken. To the best of our knowledge, role of wheatgrass as an alternative preventive therapy in colon cancer needs to be explored in experimental model, before it is recommended for clinical use. But minimum dose of wheatgrass with maximum protection has not yet been established for prevention of colon cancer in rats. Therefore, this study has been planned.

3. Materials and Methods

This study was initiated after approval from the Institutional Research Board (Animal Ethics Committee) of PGIMER vide Ref. No. 48/IAEC/230.

Sample size: Data of 6 animals in each group was collected because for animal study 6 animals in each group are accepted.

Study design: 36 male Sprague Dawley rats were split in 6 groups with each group having 6 animals. Range of body weight of the animals was 220-270g. Details of groups are given in Figure 1. These were acclimatised for 7 days prior to exposure to various treatments. Under standard conditions, they were kept in polypropylene cages. All included rats were retained in accordance and as per the guidelines and ethical principles endorsed by the committee of animal care.

Study setting: The experimental protocol was planned to decide the dose of wheatgrass as given below.

• Group 1- Throughout the period of experimentation, rats in this group were fed on standard laboratory feed, water and diet ad libitum and served as control. 1mM EDTA-saline subcutaneously per week was also administered to the rats in this group which was used as the vehicle for giving 1,2-dimethylhydrazine (DMH) treatment.

• Group 2- Rats in this group were given a weekly DMH in 1mM EDTA-saline at a dose of 30mg/kg body weight as subcutaneous injections for a total period of 16 weeks. This group was designated cancer group [14].

• Group 3- Rats in this group were given a DMH weekly in a similar way as was given to group 2 animals. Wheatgrass was administrated orally in the form of tablets (obtained from Sarvaayush wheatgrass tablets Pvt. Ltd., Pune) dissolved in water and provided in a dose of 80mg/Kg body weight daily for 16 weeks. Wheatgrass treatment was started 15 days prior to the start of DMH injections

• Group 4- Treated in same way as group 2 animals. Additionally15 days prior to the start of first DMH injection, 100mg/Kg body wheatgrass dose was administrated daily orally to animals. This dose was administered for 16 weeks.

• Group 5- DMH weekly injection were given, as was given to group 2 animals. Additionally15 days prior to the start of first DMH injection, wheatgrass was given orally to animals in water at a dose of 120mg/Kg body weight in drinking water daily for a period of 16 weeks.

• Group 6- Animals in this group were given dose of 120mg/kg body weight wheatgrass tablets daily in drinking water. The rats in this group served as wheatgrass control animals for groups 3-5. Maximum dose of wheatgrass was taken in order to check its toxicity even if ingested at high dose

After 16 weeks, animals were sacrificed using ether anaesthesia. Small colons tissue portions after weighing, were fixed in formalin for histological examination. A segment of colon was stored at -80°C and some was used for making homogenate was prepared in Tris-Mannitol buffer (2mM Tris, 50mM Mannitol, pH 7.2) for biochemical estimations. Following parameters were undertaken to establish minimum wheatgrass dose with maximum protection.

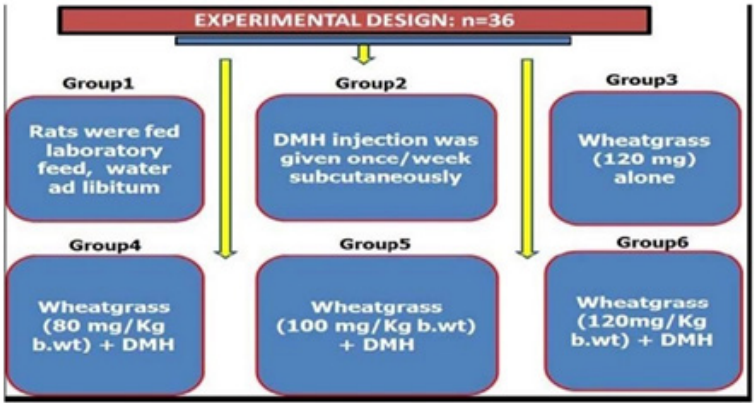

Figure 1: Showing experimental design of 36 rats divided into 6 groups (6 rats in each Group).
b.wt. = body weight, DMH = 1, 2-dimethyl hydrazine

3.1. Biochemical estimations

Activities of Superoxide dismutase (SOD), Lipid peroxidation (LPO), Catalase and Glutathione (GSH), were estimated using standard methods.

To analyse Histoarchitecture difference between control and treated rats, light microscopic studies were also done.

3.2. Statistical Analysis

All the parameters were analysed for different treatments at 16 weeks in colon of male SD rats. Various treatment group results have been compared with their corresponding controls. The statistical significance of the values was determined using analysis of variance (ANOVA). The results were expressed as mean ± SD. Statistical significance was accorded to P value <0.05.

4. Results

It was observed from table 1 that significant levels of LPO were increased (P<0.001) following administration of DMH (Group 2) and those having additionally 80mgWheatgrass along DMH (Group 3) as compared to control rats (Group 1). In contrast, GSH levels, activities of catalase and SOD were decreased significantly in rats injected with DMH only (Group 2) in comparison to control group. Those provided DMH + wheatgrass (group 3, 4 and 5) showed reduced LPO levels with increasing dosage of wheatgrass from 80mg to 100mg/ Kg body weight / day (Table 1). 100mg and 120mg dosages of wheatgrass showed significant (p<0.001) reduction in LPO as compared to DMH group but there was not much difference between the two groups. 80 mg dosage of wheatgrass showed less reduction (p<0.01) in LPO as compared to 100mg and 120mg/kg dosage of wheatgrass (p<0.001). On the other hand, activities of catalase and SOD and levels of GSH tended to show an increase following simultaneous supplementation of wheatgrass at doses ranging from 80mg to 120mg. But DMH treated rats supplemented with 80 mg of wheatgrass (group 3) did not show statistically significant changes in the activities of enzymes and GSH levels when compared with Group 2 animals (DMH only). However, enzymatic activity tended to restore with wheatgrass treatment and showed significant (P<0.05) recovery in 100mg and 120mg dose groups (P<0.05). It was also observed that recovery was almost similar in both the dose groups of 100 & 120mg. Thus, not much difference was observed in biochemical indices in supplementation of 100mg as well as 120 mg doses of wheatgrass. Thus, the wheatgrass dose was finally decided to be 100mg/kg body weight / day which provided maximum protection without causing any adverse effect.

Table 1: Effect of different doses of Wheatgrass on LPO, GSH, catalase and SOD in colon of normal and treated rats

Groups	LPO (n moles of MDA formed / mg protein)	GSH (µ moles of GSH / g tissue)	Catalase (m moles of H_2O_2 / min / mg protein)	SOD IU
Group 1: Control (n=6)	2.89±0.41	0.654±0.12	1.67±0.35	12.54± 2.09
Group 2: DMH (n=6)	5.37±0.32***	0.23±0.06 ***	0.56± 0.07 ***	5.95± 1.21 ***
Group 3: 80mgWG+DMH (n=6)	4.43±0.46$^{***,\#}$	0.29±0.06***	0.62± 0.03***	6.91± 1.08 ***
Group 4: 100mgWG+DMH (n=6)	3.34±0.31$^{*,\#\#}$	0.44±0.07 $^{*,\#}$	0.97± 0.02 $^{*,\#\#}$	8.84± 1.17 $^{**,\#}$
Group 5: 120mgWG+DMH (n=6)	3.13±0.91$^{*,\#\#}$	0.49±0.05 $^{*,\#}$	0.93±0.06$^{*,\#\#}$	8.76± 1.14$^{**,\#}$
Group 6: Wheatgrass (n=6)	2.19±0.22	0.61±0.24	1.17± 0.19	12.87± 2.23

LPO: Lipid peroxidation, GSH: reduced glutathione; SOD: superoxide dismutase; WG: wheatgrass; DMH: 1, 2-dimethyl hydrazine
80mgWG: 80mg wheatgrass / Kg body weight / day; 100mgWG: 100mg wheatgrass / Kg body weight / day; 120mgWG: 120mg wheatgrass / Kg body weight / day
Data is expressed as mean ± SD.
*P<0.05, **P<0.01, ***P<0.001 by one-way ANOVA when values of groups 2, 3, 4, 5 are compared with group 1.
#P<0.01, ##P<0.001 by one-way ANOVA when values of groups 3, 4, 5 are compared with group 2.

4.1. Histological Findings

In Figure 2, Control rats showed normal morphology (A) whereas rats treated with DMH showed well differentiation adenocarcinoma in 16 weeks (B). When DMH treated rats were supplemented with 80mg dose of wheatgrass, they showed multiple foci of severe dysplastic changes (D). However, supplementation with 100mg and 120mg doses showed appreciable improvement in histoarchitecture as occasional foci of low-grade dysplasia and inflammation was observed at selective locations (E&F). There was no dysplasia or toxicity observed in groups supplemented with wheatgrass alone(C). Normal histoarchitecture was observed in these groups as no significant changes were found and histology was comparable to that of control rats (Figure 2).

Therefore, 100mg/Kg body weight per day wheatgrass dose was observed to be a minimum dose with maximum protection, both histologically and biochemically.

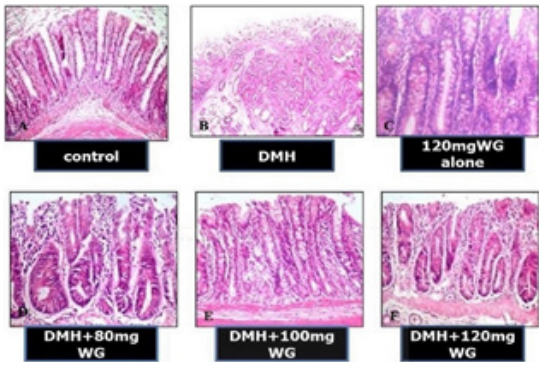

Figure 2: Showing histological representation of different groups: control (A), DMH (B), 120mg WG alone (C), DMH + 80mg WG (D), DMH+ 100mg WG (E), DMH+120mg WG (F), DMH: 1, 2-dimethylhydrazine, WG: wheatgrass.

5. Discussion

In the present study, a significant difference in lipid peroxidation was detected in the colon tissue of rats subjected to DMH treatment for 16 weeks. This increase is due to the ability of DMH to cause increase in LPO and induce formation of free radicals [15]. During treatment by DMH, there is variations in structure and function of membrane due to its effect on polyunsaturated fatty acids in biological membranes[16] via oxidative mechanisms. Decrease in GSH levels cause increased lipid peroxidation as enzymes used to detoxify peroxides contain glutathione [17]. It is reported that intracellular GSH is regulated by oncogenes and plays vital role in the growth of tumours [18]. This significant decrease in levels of GSH in consequence to increased LPO in the present study has been mirrored by the studies conducted by other workers [19, 20].

It was observed in the present study that after DMH treatment of 16 weeks, activities of catalase and SOD decreased. Decreased SOD and catalase activity may result in increase in the levels of ROS and proliferation of colonocytes as seen in CRC. This may also resemble with earlier reports, which showed that cancer in humans produces large amounts of hydrogen peroxide and utilization of catalase in converting the H2O2 to H2O may result in decreased levels [21, 22]. Moreover, decreased SOD activity, further emphasizes immense DMH induced oxidative stress. Decrease SOD activity is known to promote the cancerous tissue expansion and infiltration into the surrounding tissues [23].

Histological changes in the colons were studied in normal and treatment groups. The histopathological disturbances were seen in colon tissue following 16 weeks of DMH treatment. 16 weeks' treatment with DMH in group 2 showed well differentiated adenocarcinoma with multiple variably sized irregular glands composed of cubo-columnar cells, with enlarged hyperchromatic nuclei, loss of polarity, increased mitotic activity and moderate amount of eosinophilic cytoplasm. DMH group with matching results have also been reported in literature which highlighted that DMH is a potent colon carcinogen and caused occasional dysplasia and aggregates of lymphoid cells in the localized regions with severe hyperplasia [24]. Animal studies indicate that procarcinogen 1, 2-dimethylhydrazine (DMH) induced experimental colonic tumours share comparable histology and anatomical morphology to human colonic neoplasms [25]. It has also been observed in the present study that 0% tumour incidences were found in alone wheatgrass treated groups which suggests that wheatgrass is nontoxic since it does not cause any disruption in normal cellular homeostasis. The possible action of wheatgrass has been explained in terms of abscisic acid (ABA). ABA is present in wheatgrass which is a challenging anti-cancer agent and even in small amount is proved to be deadly against any form of cancer (Gloria, 2007). Alkaline and well-oxygenated environment does not allow cancer cells to flourish. Since wheatgrass provides an alkaline environment to the cells, it is a powerful food for cancer patient while chlorophyll oxygenates the body [26]. This may be the reason of protection of colon cancer by wheatgrass in present study.

6. Conclusion

This study shows that minimum dose of wheatgrass with maximum protection against colon cancer in rats is 100mg/kg body weight/day which can be used for further experiments.

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