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Assessment of the preventive effect of dietary inclusion of *Cucurbita* maxima (Duch.) leaf on N-methyl-N-nitrosourea (MNU) induced colon carcinogenesis in Wistar rats

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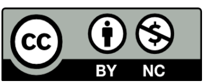

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Abstract: The preventive effect of *Cucurbita maxima* leaf inclusion at 2.5%, 5% and 10% in colon carcinogenesis induced by N-methyl-N-nitrosourea (MNU) for 12 weeks was evaluated. A significantly lower level of carcinoembryonic antigen (CEA) values was observed in the serum of rats fed with various percentages of *C. maxima* leaf included diet in comparison with the MNU control group (administered MNU without treatment), which showed high serum CEA values (238.77 ± 38.95 ng/ml). The result shows that the MNU control group has the highest level of oxidative stress in the colon (78.51 ± 6.88 nMol/mg/ml) when compared to the normal control groups (56.30 ± 5.82 nMol/mg/ml). There was no significant difference in the level of MDA in the 10% dietary inclusion control group of the colon (52.08 ± 7.63 nMol/mg/ml) when compared to the normal control groups. Analysis of the experimental diets revealed a significant presence of total polyphenols and flavonoids content which increases from 0.256 mg/g GAE and 0.068 mg/g QU respectively in basal diets towards 0.287 mg/g and 0.085 mg/g respectively in the 10% *C. maxima* leaf-included. The polyphenol and flavonoid content of the formulated diet at 5% and 10% shows significantly higher values than the basal diet. The data showed an increase in crude fibre content, 3.05 mg/g, 3.1 mg/g, 4.2 mg/g in the 2.5%, 5% and 10% inclusions respectively. The results demonstrated a strong negative correlation coefficient (-0.715, -0.799, -0.944) between CEA and the crude fibre, flavonoid and polyphenol respectively when *C. maxima* percentage was increased in the experimental diets. Similarly, the rats MDA values also showed a negative correlation coefficient (-0.271, -0.398, -0.147) with crude fibre, polyphenol and flavonoid content respectively. However, the enzymatic antioxidants (superoxide dismutase and catalase) showed a positive correlation (0.355, 0.411, 0.488 and 0.112, 0.241, 0.380) with the crude fibre, polyphenols and flavonoid content respectively. Histological observation of colon tissue showed severe damage to the mucosa cell, with mucosa ulceration and sclerosis in the MNU control group, whereas groups fed with experimental diets concurrently with MNU administration showed mild damage to the normal architecture of the cells. The results demonstrated the ability of *C. maxima* leaf dietary inclusion to improve endogenous antioxidant system, lower oxidative stress and protect against organ damage by MNU carcinogen.

Keywords: *Cucurbita maxima*; dietary inclusion; carcinogenesis; chemo-preventive; N-methyl-N-nitrosourea; MNU; colon cancer

1. INTRODUCTION

Colon cancer is now seen as a major challenge to modern medicine because it is one of the most commonly diagnosed cancers both in men and women across the globe and it has been acclaimed to be the third leading cause of cancer related death [1]. In 2012 alone, an estimated 14.1 million cases (52% men and 48% women) were reported worldwide with the expectation to increase to 24 million by the year 2035, however, the incidence of the disease was relatively low in Africa about 5 cases per 100,000 people [2]. A critical look at the Africa data showed that South Africa and West Africa have the highest and lowest incidence respectively [3]. Nigeria was previously reported to have very low to rare cases of the disease because of the high fiber diet consumed by most of the population (WHO, 2015), but on the contrary, a report indicated a rise in the incidence of the disease which was attributed to change in dietary habits of the population from fiber-rich diet to highly refined diets due to urbanization and upsurge of fast-food outlets in major cities of the country [4]. The rise in the incidence of colon cancer will require urgent measures

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including preventive measures, especially as diagnostic facilities are inadequate and chemotherapy is unaffordable and without guarantee of complete recovery [5].

In the light of the challenges in the treatment and management of colon cancer and the absence of reliable colon cancer preventive drugs, edible medicinal plants could provide the key in the prevention of this disease there is a need to investigate more naturally abundant edible plants of medicinal potency in the fight against colon cancer until total and absolute protection from colorectal cancer is guaranteed. One such diverse plant option is *C. maxima* as commonly called giant pumpkin [6]. The focus of this investigation, therefore, is to consider the possible preventive effect of the leaf of *C. maxima* formulated diet on colon carcinogenesis using N-methyl-N-nitrosourea induced male Wistar rats as a model.

2. MATERIALS AND METHODS

2.1 Plant Collection and Preparation

Fresh leaves of *C. maxima* were collected in June from Ejule in Ofu LGA of Kogi State, Nigeria, and authenticated at the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria with a Voucher Number 1077. They were rinsed rapidly under running tap water, shaken thoroughly, dried under shade, and pounded using laboratory pestle and mortar. The powdered material was kept in air-tight polyethene bags and stored at room temperature till required.

2.2 Analysis of Total Phenolic Compounds

Phenolic compounds were determined based on a method described by Fraga *et. al* [7]. Exactly 0.25 ml of 500 mg/L of sample extract was mixed with 2.5 ml of Folin Ciocalteu reagent. After 3 min, 2.0 ml of 1.0 M sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled H_2O. The reaction mixture was kept in the dark for 15 minutes at room temperature with intermittent shaking. The absorbance was measured at 765 nm using a spectrophotometer (UNICAM UV300). Phenolic contents were calculated based on the standard curve for gallic acid (GAL). The results were expressed as mg of gallic acid equivalent per gram of dry extract.

2.3 Determination of Flavonoids

Flavonoid compounds were determined based on a method described by Fraga *et. al.* [7]. Briefly, 0.5 ml of 500 mg/L solutions of each fraction of extract was added to 1.5 ml of absolute methanol, 0.1 ml of 10% aqueous $AlCl_3$, and 0.1 ml of 1M CH_3COOK was added followed by 2.8 ml of distilled water. The reaction mixture was mixed thoroughly, kept in the dark for 30 minutes at room temperature. The intensity of the pink color was measured at 415 nm using a spectrophotometer (UNICAM UV300). The level of total flavonoid concentration was calculated using quercetin (QU) as a standard. The results were expressed as mg of quercetin equivalents per gram of dry extract

2.4 Determination of Crude Fibre

The crude fiber was determined based on the methods of (Association of Analytical chemists (AOAC, 2012). The principle is based on the fact that crude fiber is lost on ignition of dried residue remaining after digestion of sample with 1.25% H_2SO_4 and 1.25% NaOH solutions under specific conditions.

2.5 Experimental Animals and Maintenance

Healthy male *albino* rats of the Wistar strain weighing 80-100g were acquired from the Animal Unit of the National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. The animals were housed in a standard plastic cage with wooden shavings as their beddings at room temperature (25ºC) with 50% humidity, and a 12-hour light-dark cycle in the Department of Biochemistry Animal House, Ahmadu Bello University Zaria and allowed free access to tap water and standard feed (vital feed grower mash produced by UAC Company Jos, Plateau, Nigeria) *ad libitum* for two weeks to acclimatize. All animals were cared for according to the institutional guidelines for the care and use of experimental animals.

2.6 Animal Grouping

At the end of the acclimatization period, all rats were weighed and randomly divided into six groups of seven animals each (n=7). The experimental animals were fed according to the group's percentage inclusion of *C. maxima* leaf in the standard diet. Group 1 received normal saline and was fed on standard diet (positive control), Group 2 received 200 µL MNU and was fed on standard diet (negative control), while Group, 3, 4, and 5 fed on 2.5%, 5%, and 10% *C. maxima* leaf-included diets respectively with concomitant intra-rectal 200 µL MNU instillation.

2.7 Induction of Colon Cancer

Exactly 0.072 g (72 mg) of N-methyl-N-nitrosourea (MNU) powder was dissolved in 6 ml of (1.9%) citric acid solution. Thereafter, rats in all the experimental groups except the normal control group received 200 µl of the freshly prepared aqueous MNU each with the aid of a tube with a cannula through intra-rectal installation administration every 72 hours for 12 weeks.

2.8 Collection of Colon Tissue

Following mild chloroform anesthesia, the animals were sacrificed by decapitation one week after the end of the 12 weeks of the experiment. The colon was dissected out quickly, washed immediately with ice-cold saline to remove bloodstain, and weighed (absolute weight) using a digital weighing balance. Section of the colon was fixed quickly in 10% normal saline for histopathological analysis and the remaining organ refrigerated for other subsequent analyses.

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2.9 Tissue Homogenization

The colon, (100 mg tissue/mL buffer) was crushed using porcelain mortar and pestle in 50 mM phosphate buffer (pH 7.4) to obtain a 10% w/v solution. The homogenate was then centrifuged at 10,000 rpm for 10 minutes and the supernatant was collected with a Pasteur pipette and immediately stored at -4 degree Celsius for subsequent analysis.

2.10 Carcinoembryonic Antigen (CEA) Assay

The concentration of CEA was determined by enzyme-linked immunosorbent assay (ELISA) which is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact CEA molecule that is used for solid-phase immobilization (on the microtiter wells). A goat anti-CEA antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample was allowed to react simultaneously with the two antibodies, resulting in the CEA molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 1 hour of incubation at room temperature, the wells were washed with water to remove unbound labeled antibodies. A solution of 3, 3’, 5’, 5-tetramethylbenzidine (TMB) reagent was added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of a drop of Stop Solution 0.16M (sulfuric acid) changing the color to yellow. The concentration of CEA is directly proportional to the color intensity of the test sample. Absorbance was measured spectrophotometrically at 450 nm using ELISA reader (GF-M30000 Microplate reader, Instrument no: 200144012, BBRAN Scientific and Instrument Company, England. The CEA concentration of the sample was calculated using the following formula. Concentration = Absorbance - Blank x standard x dilution factor.

2.11 Lipid Peroxidation Malondialdehyde (MDA) Level

The concentration of thiobarbituric acid reactive substances (TBARS) in the tissue homogenate was estimated by the method of Martin *et al.* [8]. In this procedure, 1.0 ml of 0.67% thiobarbituric acid (TBA) was added to 1.0 ml of 14% trichloroacetic acid (TCA) and 50 µL of the supernatant of tissue homogenate, and the mixture was shaken thoroughly. The reaction mixture was then incubated in a water bath at 80ºC for 30 minutes. After the incubation, the solution was cooled in ice-cold water for 10 minutes and centrifuged at 3000 rpm for 10 minutes before the supernatant was collected and the absorbance read at 535 nm against the reagent blank. The blank preparation was performed according to the procedure above except that the sample was replaced with an equal volume of distilled water. The concentration of TBARS is expressed in terms of Malondialdehyde (MDA) in µM. Molar extinction coefficient of MDA is 1.56 x $10^5 cm^{-1}m^{-1}$.

MDA concentration = Absorbance/ 1.56 x $10^5 cm^{-1}m^{-1}$

2.12 Superoxide Dismutase (SOD) Activity

The enzyme, superoxide dismutase (SOD) decomposes superoxide anion into hydrogen peroxide and oxygen at a high reaction rate. Thus, the SOD activity assay method is based on monitoring the auto-oxidation rate of hematoxylin in the presence of SOD as described by Aebi [9]. This is based on the principle that in the presence of the SOD enzyme, the rate of auto-oxidation is inhibited and the percentage of inhibition is linearly proportional to the amount of SOD present within a specific concentration range. Sample SOD activity is determined by measuring ratios of auto-oxidation rates in the presence and absence of the sample. The procedure involved the addition of a 40 µL sample to 920 µL of phosphate buffer (0.05 M, pH 7.8), while the reagent test was prepared by replacing the sample with 40 µL of normal saline. The mixture was incubated for 2 minutes at 25ºC before the addition of 40 µL of hematoxylin, following which absorbance of the mixture was read at 560nm immediately and after 5 minutes against the sample blank. Enzyme activity calculated using the formulae:

$$U/_{min} = \frac{A1 - A2}{t1 - t2}$$

whereA1= initial absorbance
A2=final absorbance
t1=initial time
t2=final time

Concentration was obtained using the following formulae:

$$A = ECL$$

where C=concentration, A=absorbance, E=molar extinction coefficient, and L=path length of the Cuvette

2.13 Catalase Activity Assay

Catalase activity was determined according to Nozoe *et al.* method [10] by monitoring the decomposition of hydrogen peroxide (H_2O_2). The principle for this assay was based on the assumption that the enzyme. catalase, tend to facilitate the breakdown of hydrogen peroxide into water and oxygen, therefore the rate of consumption of the hydrogen peroxide in the sample medium is directly proportional to the activity of the enzyme present. The tissue homogenate, 10 µL was added to 2.8 ml of 50 mM potassium phosphate buffer (pH 7.0) while 100 µL of 30 mM H_2O_2 was added to initiate the reaction. The absorbance of the reaction mixture was each minute taken at 240 nm for 5 minutes and H_2O_2 consumption was calculated using extinction coefficient, 39.4 $mM^{-1}cm^{-1}$. Catalase activity was determined and expressed as (U/ml) from the decomposition rate given as ($\Delta A_{240nm/min}$) of the sample.

$\Delta A_{240nm/min}$ = Change in absorbance per minute.

Catalase (U/ml) = ($\Delta A_{240nm/min}$)/ Volume of reaction mixture

2.14 Histological Analysis of Colon

The organ tissues were rapidly fixed for 18-24 hours in 10% formalin and dehydrated in ascending grades of alcohol (70%, 80%, 90%, 95%, and 100%) for 2 hours each. The

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tissue was cleared in xylene and subsequently transferred into a pot of melted paraffin for additional 2 hours (infiltration). It was then immersed in a mold containing molten paraffin wax, which was allowed to solidify (embedding), thus preparing the tissue for sectioning. Tissue sections of 3 μm in thickness were prepared according to standard micro techniques onto glass slides and stained with hematoxylin and eosin before photomicrographs were obtained under the light microscope at magnifications (10x) for histological changes.

2.15 Statistical Analysis

The statistical significance between the controls and other groups of the experimental animals was determined by the two-way ANOVA followed by Duncan's multiple comparison tests using SPSS version 20. Statistical test was performed at $p<0.05$ level of significance with the result reported as Mean ± SD.

2.16 Ethical Consideration

All ethical matters as concerned animals handling were observed following the animal ethical rules of the Department of Biochemistry, Ahmadu Bello University Zaria.

3. RESULTS

3.1 Effect of *C. maxima* Leaf Dietary Inclusion on Serum Carcinoembryonic Antigen (CEA) Level

The effect of dietary *C. maxima* leaf inclusion on the CEA levels of the experimental animals in various groups chronically administered MNU intra-rectally for 12 weeks is presented in table 1. The result showed a significantly ($p<0.05$) higher CEA level in the MNU control group when compared with the normal control group and dietary control group. However, there was a dose-dependent decrease in the CEA value of the treatment groups when the percentage inclusion of *C. maxima* leaf was increased from 2.5% to 10%, and no significant ($p<0.05$) differences between the normal control and dietary control group.

3.2 Effect of *C. maxima* Leaf Dietary Inclusion on MNU-Induced Lipid Peroxidation

The level of lipid peroxidation in the colon of the experimental animals administered MNU carcinogen intra-rectally for 12 weeks at different levels of dietary inclusion is shown in table 1. The result shows that the MNU control group has the highest level of oxidative stress ($p<0.05$) in the colon (78.51 ± 6.88) when compared to the normal control groups (56.30 ± 5.82) There was a lower (not statistically significant at $p<0.05$) level of MDA in the dietary inclusion control group of the colon (52.08 ± 7.63) when compared to the normal control groups.

Table 1. Effects of *C. maxima* Leaf Inclusion Diet on Carcinoembryonic Antigen (CEA) concentration Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase, and Mismatch Repair Gene (MMR) in Rats Colon Administered N-methyl-N-nitrosourea Intra-rectally, for 12 weeks

Treatment	CEA level (ng/ml)	MDA (nMol/mg/ml)	SOD (U/min/mg protein)	Catalase (U/min/mg Protein)	MMR Gene (%)
Basal diet control	122.50 ± 20.55^{a}	56.30 ± 7.8^{a}	237.00 ± 17.3^{a}	3.133 ± 0.4^{a}	75 ± 2.3^{a}
MNU control	238.77 ± 38.95^{b}	78.51 ± 8.9^{b}	145.67 ± 11.9^{b}	1.433 ± 0.1^{b}	37 ± 0.4^{b}
MNU + 2.5% *C. maxima*	160.91 ± 20.59^{c}	70.64 ± 13.2^{b}	141.00 ± 5.3^{b}	1.833 ± 0.2^{b}	42 ± 0.4^{b}
MNU + 5% *C. maxima*	160.21 ± 13.52^{c}	67.94 ± 10.0^{c}	222.33 ± 3.7^{a}	4.667 ± 0.5^{c}	65 ± 1.2^{c}
MNU + 10% *C. maxima*	140.09 ± 20.93^{d}	56.47 ± 7.8^{a}	333.00 ± 18.6^{a}	4.267 ± 0.1^{c}	81 ± 3.1^{a}
10% *C. maxima*	122.3 ± 26.00^{a}	52.07 ± 7.6^{a}	251.67 ± 18.1^{c}	6.933 ± 0.7^{d}	90 ± 3.6^{d}

n=6, values are in mean ± standard deviation; values with different superscript down the column are significantly different at ($P<0.05$).

3.3 Effect of *C. maxima* Leaf Dietary Inclusion on Superoxide Dismutase (SOD) Activity in the Rat Colon

The superoxide dismutase (SOD) activity in colon homogenate of the experimental animals administered MNU concurrently fed *C. maxima* leaf dietary inclusion is shown in table 1. The result shows a significantly ($p<0.05$) lower SOD activity in the colon of the MNU control group when compared to the normal control groups and the MNU groups fed included-diet groups. There was a significant difference between the SOD activity colon of diet control and the group fed the highest percentage of *C. maxima*. However, when compared with the normal control, there is no statistical ($p<0.05$) difference. Greater SOD activity was observed in the colon with an increasing level of *C. maxima* leaf inclusion.

3.4 Effect of *C. maxima* Leaf Dietary Inclusion on Catalase Activity in the Colon of MNU Administered Rats

The result shows a significantly ($p<0.05$) higher catalase activity in the colon homogenate of the group that took the dietary inclusion without MNU induction when compared to the normal control (table 1). However, the MNU induced but the untreated group had a significantly ($p<0.05$) lower catalase activity when compared with the normal control and treatment groups.

3.5 Total Polyphenol, Flavonoid and Crude Fiber Content in *C. maxima* Leaf Included-diets

Analysis of the experimental diets revealed a significant presence of total polyphenols and flavonoids content as depicted in table 2. The polyphenol and flavonoid content of the formulated diet at 5% and 10% shows significantly higher values than the basal diet however, they were lower than that of the MNU group diet. Also presented is the data for the crude fiber content of the experimental diets, which showed an increase in crude fiber content, 3.05 mg, 3.1 mg, and 4.2 mg in the 2.5%, 5%, and 10% inclusions respectively.

3.6 Correlation Analysis for Various Experimental Parameters

The correlation of various experimental parameters with crude fiber, total polyphenol, and flavonoid content of diets included with *C. maxima* leaf at 0%, 2.5%, 5%, and 10% are shown in table 3. The result shows a strong negative correlation coefficient (-0.715, -0.799, -0.944) between CEA and the crude fiber, flavonoid, and polyphenol respectively when *C. maxima* percentage was increased in the experimental diets. Similarly, the rats' MDA values also showed a negative correlation coefficient (-0.271, -0.398, -0.147) with crude fiber, polyphenol, and flavonoid content respectively. However, the enzymatic antioxidants (Superoxide dismutase and catalase) showed a positive correlation (0.355, 0.411, 0.488 and 0.112, 0.241, 0.380) with the crude fiber, polyphenols, and flavonoid content respectively.

3.7 Effect of Dietary Inclusion of *C. maxima* Leaf on Histology of the Colon Tissue of Rats

The histological section of the colon in various treatment groups administered MNU intra-rectally and fed various levels of *C. maxima* leaf included diets for 12 weeks are shown in figure 1. The MNU control group (B) showed colon tissue with severe mucosal ulceration, sclerosis, and inflammation, these effects were prevented to different degrees depending on the *C. maxima* leaf with the 10% included diet showing the best effects, having essentially normal colon cell architecture as the normal control group.

Table 2. Polyphenol, flavonoid and crude fiber content of *C. maxima* leaf dietary inclusion.

Sample	Polyphenol (mg/gGAE)	Flavonoids (mg QU/g)	Crude fiber (mg/g)
Basal diet control	0.256 ± 0.01	0.068 ± 0.01	5.87 ± 0.8
MNU control	0.444 ± 0.06	0.155 ± 0.09	2.38 ± 0.4
MNU + 2.5% *C. maxima*	0.264 ± 0.05	0.038 ± 0.01	3.05 ± 0.2
MNU + 5% *C. maxima*	0.323 ± 0.01	0.074 ± 0.03	3.1 ± 0.01
MNU + 10% *C. maxima*	0.287 ± 0.02	0.085 ± 0.02	4.2 ± 0.5

n = 3, values are in mean± standard deviation

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Table 3. Correlation values of CEA, MDA, catalase, SOD, crude fiber, total polyphenols and flavonoids

Parameters	CEA	MDA	SOD	CAT	MMR Expression	Polyphenol	Flavonoids	Fiber
Carcinoembryonic antigen (CEA)	1							
Malondialdehyde (MDA)	0.492	1						
Superoxide dismutase (SOD)	-0.788	-0.752	1					
Catalase (CAT)	-0.688	-0.755	0.978	1				
MMR expression	-0.530	-0.281	0.428	0.401	1			
Total polyphenols	-0.944*	-0.147	0.488	0.380	0.472	1		
Total flavonoids	-0.799*	-0.398	0.411	0.241	0.635	0.929**	1	
Fiber	-0.157	-0.271	0.355	0.112	0.212	0.617	0.401	1

1 is a perfect correlation. Values with negative signs show correlation in inverse direction.
** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

4. DISCUSSION

Carcinoembryonic antigen is a glycoprotein with a molecular weight of approximately 180 kDa which is normally present at only very low concentrations in adult plasma, but its concentration can be significantly increased during the process of carcinogenesis or in the presences of tumors particularly colorectal cancers [11]. It is a well-known biomarker used to determine the extent of preoperative colon cancer [12]. Thus, CEA concentration was significantly higher in the MNU control group compared to the normal control group and MNU groups fed experimental diets suggesting the onset of colon carcinogenesis's reported by Ure *et al.* [13] who demonstrated overexpression of CEA whenever there is cancer development. The inverse relationship seen between CEA and percentage dietary inclusion with the leaf of *C. maxima* could be attributed to the high antioxidant boosting the ability of the plant due to the presence of high polyphenols and flavonoids and by extension prevented carcinogenesis. Similarly, Zeng *et al.* in their recent work showed that markers of oxidative stress tend to show a strong positive correlation with CEA [14], this implies that substance that reduces oxidative stress may lower the CEA values, as can be seen when the percentage of inclusion of the *C. maxima* leaf were increased. Experimental evidence suggests that *C. maxima* leaf contains a significant amount of crude fiber which might have helped in reducing contact time between the colon cells and carcinogen thus preventing initiation of cancer and also may be the reason for the observed lower CEA values. Furthermore, the crude fiber in the leaves of *C. maxima* might be converted to butyrate which is an inhibitor to tumor growth [15], possibly explaining the reason for the low CEA value, especially in the dietary control groups. A similar contribution put forward by other scientists is that crude fiber resists digestion in the small intestine and thereby enters the large intestine where it is fermented to produce short-chain fatty acids which have anticancer properties [16]. Also, apart from increasing antioxidant yield, they tend to increase fecal bulking and viscosity thereby decreasing "contact time" between carcinogens ingested and mucosa cells of the large intestine, preventing cancer initiation [17]. The shift in the balance between oxidants and antioxidants in favor of the oxidant is term oxidative stress. Oxidative stress and lipid peroxidation can be estimated by determining the activity of endogenous antioxidant enzymes and thiobarbiturate acid reactive substances (malondialdehyde MDA, 4-hydroxynonenal HNE) respectively [18, 19]. The significant higher ($P<0.05$) levels of malondialdehyde (MDA) in the rat colon as seen in MNU groups and 2.5% may be as a result of an enhanced membrane lipid peroxidation by free radicals generated by the MNU and the failure of the *antioxidant* defense mechanisms in the system to reduce the excessive formation or detrimental effects of such free radicals probably because the endogenous antioxidants enzymes have been overwhelmed [20], by the continuous exposure to MNU carcinogen. Similarly, continuous exposure might initiate carcinogenesis which in turn generates more oxidants and the over when the antioxidants system [21]. The low level of oxidative stress and lipid peroxidation with increasing level of dietary inclusion with the leaf of *C. maxima* could be attributed to the high free radical scavenging potential of the plant leaf as reported by Ganie *et al.* [22], presumably due to the total polyphenols and flavonoids content, as reflected in the inverse relationship between these parameters and that of the oxidative stress -0.398, -0.147 respectively (table 3).

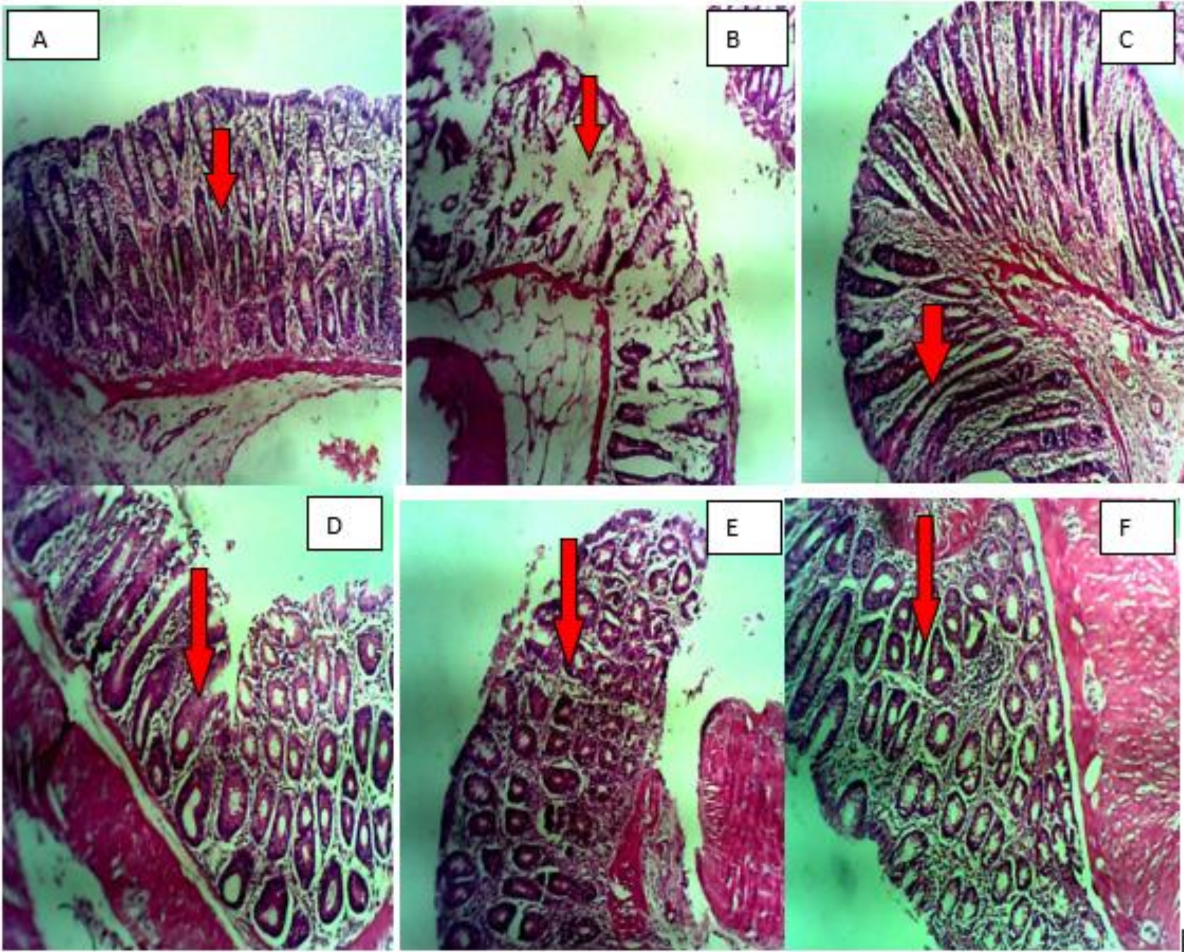

Figure 1. Representative photomicrographs in colon tissue section of rats administered MNU intra-rectally and fed with corresponding *C. maxima* leaf inclusion diet for 12 weeks; (A) Normal control group and (B) MNU control group showing a severe ulceration specifically deeper mucosal ulceration(C)) 2.5% inclusion with *C. maxima* leaf induced with MNU showing moderate sclerosis of the mucosa glands, (D) 5% inclusion with *C. maxima* leaf induced with MNU shows mild ulceration and moderate inflammation within the cells. (E) 10% inclusion with *C. maxima* leaf induced with MNU showing mild ulceration and moderate inflammation within the mucosa glands. (F) 10% inclusion with *C. maxima* leaf without MNU showing essentially normal colon with mucosa gland intact. (STAIN: haematoxylin and eosin; Magnification = 10x).

The significantly decreased ($P<0.05$) activities of endogenous antioxidant enzymes (SOD and CAT) specifically in the colon of the MNU control group when compared with the normal control may be attributed to the high concentration of these free radicals generated by the MNU during the 12 weeks of administration which may have led to decreased level or inactivation (adaptive response) of these endogenous antioxidant enzymes [23]. Feeding with dietary inclusion of the leaf of *C. maxima* at 10 % as well as 10 % dietary control restored levels of SOD and CAT activities to normal., suggesting that *C. maxima* leaf possess free radical scavenging capacity due to the presence of antioxidant especially polyphenol (phenolic acid and flavonoids) and flavonoid (flavones, flavanol and proanthocyanidins (table 2) [24], which positively correlated with the boosting of endogenous antioxidant enzyme level (table 3). The histopathological changes including (ulceration, inflammation sclerosis, etc.) in the colon of the MNU-intoxicated control group indicated that the carcinogen MNU caused severe damage to the mucosa cells. However, maintenance of colon cells architecture observed following the feeding of various percentages of *C. maxima* leaf- included diet suggest a protective effect of some *C. maxima* leaf constituents, presumably due to total polyphenol and flavonoid which significantly boosted endogenous antioxidant enzyme level, thereby preventing damage to normal cellular macromolecules such as lipids and protein [25]. However, flavonoids and polyphenols have also been found to have anti-inflammatory effects by biochemically inhibiting enzymes such as xanthine oxidase, Ca (+2)-ATPase, and cyclooxygenase [26]. The capacity of *C. maxima* constituents to scavenge free radical has been reported by Dhingra *et al.* [27], and evident from the inverse relationships between phytochemicals such as polyphenol, flavonoids, and MDA (-0.147, -0.398), index of oxidative stress (SOD and Catalase) respectively (0.488, 0.411 and 0.380, 0.242). Thus, the inclusion of *C. maxima* leaf at 5% and 10% level which were seen to have significant amounts

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of bioactive polyphenols and flavonoids may have exerted beneficial effects through its antioxidant capacity against pathophysiological alterations caused by the presence of superoxide and hydroxide as well as hydrogen peroxide radicals, this, therefore prevent damage to the colon tissue, these might be presumably due to the antioxidants the plant possess, that can scavenge free radicals. The negative correlation between the crude fiber content of the experimental diets and the CEA value (-0.715) suggests the beneficial effect of crude fiber in colon cancer chemoprevention presumably by reducing the transit time of the carcinogen in the gastrointestinal tract (GIT), and hence preventing colon carcinogenesis [27]. Polyphenols and flavonoids have been found to have health benefits by fighting cancer, free radicals, inflammation, and oxidative stress [28]. Their contents in the inclusion diets showed a negative correlation with MDA levels (-0.247, -0.398) in the colon of the rats in a dose-dependent manner with the percentage of inclusion (table 3). This might be attributed to the antioxidant capacity of these phytochemicals to prevent oxidative stress. The positive correlation between the SOD, catalase and crude fiber, flavonoids, polyphenol is additional evidence to suggest that the inclusion rate of *C. maxima* leaf in the diet of animals might have a significant effect on the endogenous antioxidant system of the experimental animals [29].

5. CONCLUSION

The results from this study demonstrated the chemo-preventive potential of the leaf of the *C. maxima* plant. Upon increasing the % inclusion of the leaf in the animal diet, the MDA and CEA values tend to decrease, while the SOD and Catalase got improved suggesting that the plant leaf contains some bioactive constituents like polyphenols, flavonoids, and even crude fiber that exerted protective effects on these parameters. It is seen from the results that Polyphenols with coefficient (r) values of -0.944, -0.147, 0.488, and 0.380 as correlated with CEA, MDA, SOD, and catalase parameters respectively tend to provide a better role in colon cancer chemoprevention when concomitantly compared to crude fiber values of -0.75, -0.27, 0.355 and 0.112 for CEA, MDA, SOD, and Catalase respectively. These results demonstrated from all the aforementioned that, dietary inclusion with *C. maxima* leaf can provide a significant chemo-preventive effect against chemically-induced colon carcinogenesis, presumably as a result of their constituents of polyphenols, flavonoids, and crude fiber.

Declarations

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References

[1] American Cancer Society (2015). *Cancer Facts & Figures, Atlanta*, GA: American Cancer Society. https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2015.html [Accessed: September 06, 2021].

[2] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathors C, Rebello M, Parkin DM, Forman D, Bray F (2013). Cancer incidence and mortality worldwide: sources, methods and major patterns in (GLOBOCAN 2012). *Int J Cancer*; 136(5):359-386. [CrossRef]

[3] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011). Global cancer statistics. *CA: A Cancer J Clinic*; 61(2):69–90. [CrossRef] [PubMed]

[4] Siegel RL, Miller KD, Jemal A (2015). Cancer statistics. *CA: A Cancer J Clinic*; 65(1):5-29. [CrossRef] [PubMed]

[5] Muntean E, Muntean N, Duda M (2013). *Cucurbita maxima* Duch. as a medicinal plant. *Hop and Medicinal Plants*; 21(1-2):75-80.

[6] Lachman J, Proněk D, Hejtmankova A, Dudjak J, Pivec V, Faitová K (2003). Total polyphenol and main flavonoid antioxidants in different onion (*Allium cepa* L.) varieties. *Hort Sci*; 30(4):142–147. [CrossRef]

[7] Fraga CG, Leibovitz BE, Toppel AL (1988). Lipid peroxidation measured as TBARS in tissue slices: characterization and comparison with homogenates and microsomes. *Free Radic Biol Med*; 4(3):155-161. [CrossRef] [PubMed]

[8] Martin JP, Dailey M, Sugarman E (1987). Negative and positive assays of superoxide dismutase based on haematoxylin autoxidation. *Arch Biochem Biophys*; 255(2):329-336. [CrossRef] [PubMed]

[9] Aebi HE (1983). Catalase. In: Bergmeyer HU (Ed) *Methods of Enzymatic Analysis, Verlag Chemie, Weinhem*: 273-286.

[10] Nozoe T, Saeki H, Sugimachi K (2001). Significance of preoperative serum C-reactive protein as an indicator of prognosis in oesophageal carcinoma. *Am J Surg*; 182(2):197-201. [CrossRef] [PubMed]

[11] Nakatani H, Kumon T, Kumon M, Hamada S, Okanoue T, Kawamura A, Nakatani K, Hiroi M, Hanazaki K (2012). High serum levels of both Carcinoembryonic antigen and Carbohydrate antigen 19-9 in a patient with sigmoid colon cancer without metastasis. *J Med Invest*; 59(3-4):280-283. [CrossRef] [PubMed]

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[12] Screaton RA, DeMarte L, Dráber P, Stanners CP (2000). The specificity for the differentiation blocking activity of carcinoembryonic antigen resides in its glycophosphatidyl-inositol anchor. *J Cell Biol*; 150(3):613-26. [CrossRef] [PubMed]

[13] Ure O, Ata N, Kucukazman M, Tutal E, Caglar F, Hokkaomeroglu M, Akin K, Dal K, Yilmaz H, Kizilca G, Beyan E, Ertugrul D (2012). Glycaemic control influence CA-19-9, CEA and ferritin levels in type 2 diabetes. *Endocrine Abstract*; 29:512.

[14] Zeng H, Lazarova DL, Bordonaro M (2014). Mechanism linking dietary fibre, gut microbiota and colon cancer prevention. *World J Gastrointest Oncol*; 6(2):41-51. [CrossRef] [PubMed]

[15] Young GP, Hu Y, Leu RKL, Nyskohus L (2005). Dietary fibre and colorectal chance, A model for environment - gene interaction. *Mol Nutr Food Res*; 49(6):571-584. [CrossRef] [PubMed]

[16] Lattimer JM, Haub MD (2010). Effects of dietary fiber and its component on metabolic health. *Nutrients*; 2(12):1266-1289. [CrossRef] [PubMed]

[17] Abdel-Wahhab KG, Mannaa FA, Abdel-Wahhab MA (2013). The protective effects of whey protein and spirulina against CCL4-induced erythrocyte damage in rats. *J Appl Sci Res*; 9(3):2063-2071.

[18] Obidah W, Garba GK, Fate JZ (2011). Protective effect of *Bixa orellena* seed oil on carbon tetrachloride induced liver damage in rats. *Rep Opin*; 3(1):92-95.

[19] Kim H-J, Kong M-K, Kim Y-C (2010). Beneficial effects of Phellodendri Cortex extract on hyperglycaemia and diabetic nephropathy in streptozotocin-induced diabetic rats. *BMB Rep*; 41(10):710-15. [CrossRef] [PubMed]

[20] Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O (2012). Oxidative stress and antioxidant defence. *World Allergy Organ J*; 5(1):9-19. [CrossRef] [PubMed]

[21] Panda M, Biswas S, Bhattacharya S, Pande JN (2014). Thin layer chromatographic studies and in vitro free radical scavenging effects of *C. maxima* leaf extract. *Pharmacol Onl*; 3:1266-1272.

[22] Ganie SA, Haq E, Hamid A, Qurishi Y, Mahmood Z, Zargar BA, Masood A, Zargar MA (2011). Carbon tetrachloride induced kidney and lung tissues damage and oxidative activities of aqueous rhizome extract of *Podophyllum hexandrum*. *BMC Complement Altern Med*; 11(17):1472-6882. [CrossRef] [PubMed]

[23] Tsao R (2010). Chemistry and Biochemistry of Dietary Polyphenol. *Nutrients*; 2:1231-1246. [CrossRef] [PubMed]

[24] Ongom PA, Kijjambu SC (2013). Antibiotic prophylaxis in colorectal surgery: evolving trends. *J Mol Pharm Org Process Res*; 1(3):109. [CrossRef]

[25] Rathee P, Chaudhary H, Rathee S, Rathee D, Kumar V, Kohli K (2009). Mechanism of action of flavanoids as anti-inflammatory agent: a review. *Inflamm Allergy Drug Targets*; 8(3):229-235. [CrossRef] [PubMed]

[26] Yongabi KA, Fon EF, Lukong H, Chia PN (2014). A preliminary assessment of *C. maxima* leaves from Cameroon on haematological parameters in albino rats. *J Mol Pharm Org Process Res*; 2(3):117. [CrossRef]

[27] Dhingra D, Michael M, Rajput H, Patil RT (2012). Dietary Fibre in food: a review. *J Food Sci Technol*; 49(3):255-266. [CrossRef] [PubMed]

[28] Fridlender M, Kapulnik Y, Koltai H (2015). Plant derived substances with anti-cancer activity: from folklore to practice. *Front Plant Sci*; 6(799): 1-9. [CrossRef] [PubMed]

[29] Batra P, Sharma AK (2013). Anticancer potential of flavonoids: recent trends and future perspectives. *3 Biotech*; 3(6):439-459. [CrossRef] [PubMed]

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