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Protective Effect of Ethanol Extract of *Annona Muricata* Leaves on Hematological Profile, Histology and Oxidative Stress in 1,2-Dimethylhydrazine-induced Colorectal Carcinogenesis in Rats

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ABSTRACT: Cancer starts when cells in the body start to grow out of control (malignant growth). Colorectal cancer starts in the colon or the rectum. *Annona Muricata* is a medicinal plant and is considered as a basis for health preservation and care worldwide. Colorectal cancer has reached epidemic proportions and is considered as a serious health problem, therefore treatment and cure of this disease is of great clinical importance. 1,2 dimethylhydrazine (DMH) is a potent carcinogen for colon cancer in rats. In this study, rats were randomly allocated into six groups: control, extract alone, DMH, extract and DMH together, extract before DMH, DMH before extract. DMH was injected subcutaneously at 25mg/1000g body weight once a week for 16 weeks, while the ethanol extract of *Annona muricata* leaves (EEAML) were administered to rats orally at 120mg/1000g. After sacrifice, colon and liver sections were dissected out for histological and some biochemical analysis. Blood was also collected in EDTA bottles for hematological assays. The group injected with DMH (subcutaneously) and orally administered leaves extract of *A. Muricata* simultaneously showed similar results with the rats fed with normal feed, as there was absence of polyps in the rats' colon when compared with those injected with DMH alone for 16 weeks. Malondialdehyde level of rats injected with DMH were significantly increased ($p < 0.05$) compared to the control. Hematology indices and caspase 3 activity show the protective effects of EEAML against DMH.

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Colorectal cancer had a low incidence several decades ago. However, it has become a predominant cancer and now accounts for approximately 10% of cancer-related mortality in western countries. The environment and genetics have been implicated in the etiology of colorectal cancer (Claudia *et al*., 2009). Much is known about the etiologies of colorectal cancer (CRC), and two critical factors in CRC carcinogenesis are oxidative stress, which is regulated by balancing the production and removal of reactive oxygen and nitrogen species, and inflammation (Eboh *et al*., 2015). These two factors can participate in feedback loops in which carcinogenic steps from initiation to promotion/ progression are repeated over and over again, augmenting the process of carcinogenesis (Ilan et al., 2010). 1, 2-dimethylhydrazine (DMH) is a toxic environmental pollutant, which was reported as a specific colon procarcinogen (Claudia *et al*., 2001). Animal studies showed that experimental colonic tumors induced by DMH were of epithelial origin with a similar histology, morphology and anatomy to human colonic neoplasms. This pro-carcinogen could thus provide an adequate model for studying colorectal cancer (Wang *et al*., 2004). DMH falls in the category of an indirect inducer drug. It has the ability to

promote DNA hypermethylation of colorectal epithelial cells. AOM is a derivative of dimethylhydrazine However, unlike DMH, AOM falls under the category of a direct inducer, without relying on conversion in vivo (Bird and Good, 2001). The DMH is believed to form active intermediates including azoxymethane and methylazoxymethanol in the liver, which are transported subsequently into the colon via bile and blood. Methylazoxymethanol is decomposed to form methyldiazonium ions, which methylate cellular components (Veeresh *et al*., 2016). Natural products, especially those derived from plants, have been used to help mankind sustain its health since the dawn of medicine (Olude *et al*., 2020). The long history of employing natural products in ethnomedicine with low-prices and limited side effects, in contrast to expensive synthetic drugs with severe adverse side effects, was the main reason for the development of new pharmaceutical drugs from natural sources. *Annona muricata* , commonly known as soursop, graviola, guanabana, paw-paw and sirsak, is a member of the Annonaceae family comprising approximately 130 genera and 2300 species (Omoregie *et al*., 2020). The fruit is used as natural medicine for arthritic pain, neuralgia, arthritis, diarrhea, dysentery, fever, malaria, parasites, rheumatism, skin rashes and worms, and it is also eaten to elevate a mother's milk after childbirth. The leaves are employed to treat cystitis, diabetes, headaches and insomnia (Soheil *et al*., 2015). The present study was designed to evaluate the chemopreventive properties of EEAML on the development and growth in 1,2, dimethyl hydrazine (DMH) induced colorectal cancer in rats by analyzing the activity of caspase 3, malondialdehyde level, and some hematological parameters.

MATERIALS AND METHODS

Plant sample collection and preparation: The leaves of *Annona Muricata* were obtained from a forest area, Egor Local Government Area of Edo State and were authenticated by Dr. Akinnibosun, H.A. Voucher specimen was kept in the herbarium with voucher number UBH_A3-56 at the Department of Plant Biology and Biotechnology, University of Benin, Benin City.

The leaves were air dried for 21 days at the Department of Biochemistry University of Benin, laboratory and later pulverized into powdery form at the Department of Pharmacy, pharmacognosy laboratory in University of Benin. 550g of the powdered leaves was soaked in 5.5litres of absolute ethanol for 72 hrs with periodic stirring. Extracts were concentrated over a rotary evaporator, freeze-dried and stored in an airtight container.

Experimental design and animal treatment: Thirty-six (36) male -albino wistar rats 100-200g) were purchased and taken to the animal house at the Department of Biochemistry Faculty of Life Science, University of Benin and were weighed. The animals were housed in clean disinfected cages and initially acclimatized with standard feed (chikun grower pellets) and clean water for 2 weeks. The rats were randomly allocated into six groups, Six (6) rats to a cage. Group one received grower mash only (control) while group 2 were given daily oral dose of 120 mg kg body weight of *Annona muricata* extract all through plus feed for 16 weeks. The third group took DMH (25 mg/kg body weight once a week) only and fed for 16 weeks. Group 4 received a daily oral dose of the extract (120 mg kg body weight of *Annona muricata*) and DMH (25 mg/kg body weight once a week) for 16 weeks. Group 5 was treated initially with a daily oral dose of extract (120 mg kg body weight of *Annona muricata)* for 4 weeks followed by DMH (25 mg/kg body weight once a week) for another 12 weeks. The last group initially received DMH (25 mg/kg body weight once a week) for 12 weeks then, followed by extract (120 mg kg body weight of *Annona muricata)* for another 4 weeks. DMH was given subcutaneously.

Body weight measurement: The experimental animals were weighed before the onset of the study and this was repeated on a weekly basis. The average weight gain and loss were estimated from the values obtained from each group.

Collection of Blood: After 16 weeks, the animals were fasted overnight and sacrificed. Blood samples were drawn from the heart. The blood meant for hematological parameters was dispensed into an EDTA sample bottle, while the blood for Cysteine-Aspartic Proteases 3 (CASPase 3) was dispensed into a plain bottle. Dissected pieces of liver and colon were rinsed, homogenized in cold normal saline solution and used immediately for catalase and lipid peroxidation assay.

Biochemical assays:

Caspase 3 activity: Elabscience® Rat CASP3 (CASPASE 3) Elisa Kit was used for this assay. The ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Rat CASP3. Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Rat CASP3 and Avidin-Horseradish Peroxidase (HRP) conjugate were added successively to each microplate well and incubated. Free components were washed

away, the substrate solution was added to each well. Only those wells that contain Rat CASP3, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm. The OD value is proportional to the concentration of Rat CASP3. The concentration of Rat CASP3 in the samples was calculated by comparing the OD of the samples to the standard curve.

Assay Procedure for Sandwich-Elisa: Add 100 µL of standard or sample to each well. Incubate for 90 minutes at 37^0C. Remove the liquid. Add 100 µL of Biotinylated Detection Ab/Ag. Incubate for 1 hour at 37^0C. Aspirate and wash for 3 times. Add 100 µL of HRP Conjugate. Incubate for 30 minutes at 37^0C. Aspirate and wash for 3 times. Add 100 µL of HRP Conjugate. Incubate for 30 minutes at 37^0C. Aspirate and wash for 5 times. Add 90 µL of Substrate Reagent. Incubate for 15 minutes at 37^0C Add 50 µL of stop solution. Determine the OD value at 450 nm immediately.

Lipid peroxidation assay: The assay method of Buege and Aust, (1978) will be adopted. Malondialdehyde which is formed from the breakdown of polyunsaturated fatty acids serves as a convenient index for the determination of the extent of lipid peroxidation reaction. Malondialdehyde has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a purple or red complex that absorbs at 535 nm.

Catalase assay: The method of Cohen *et al*., (1970) will be adopted. Catalase is present in nearly all animal cells, plants and bacteria and acts to prevent accumulation of noxious H_2O_2 which is converted to O_2 and H_2O. The decomposed hydrogen peroxide is measured by reacting with excess of potassium tetraoxomanganate (vii), $KMNO_4$ and residual $KMNO_4$ is measured spectrophotometrically at 480 nm.

Hematology: Horiba ABX 80 hematology analyser was used for the determination of hematological parameters following the manufacturer's instructions.

These include hemoglobin, packed cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, white blood cell count, neutrophils, monocytes, lymphocytes, eosinophils, basophils and platelet were also analyzed.

Tissue processing procedure for histopathology: The tissues were dehydrated in ascending grade of alcohol 98% absolute alcohol (with ATP) and it was then clear in xylene (dealcoholization), it was Impregnated in molten paraffin wax also embedded with paraffin wax for proper support on the tissue cassette then it was sectioned onto a slide with the microtone and it was allowed to dry on the hot plate and it was then stained. The hematoxylin and eosin staining procedures was carried out the Dewax section in xylene (2 changes 5 minutes each) then it was hydrated in descending grades of alcohol (absolute 98%) and the section was taken to water and the tissue samples were stained by hematoxylin for 10 minutes and then rinsed in water and further differentiated briefly in 17% acid alcohol it was blue in warm water in 10 minutes and also was counter stained with eosin for 1-3 minutes and also rinsed in water and was then dehydrated in ascending grade of alcohol (3 changes) for 5 minutes each and then finally mounted in DPX the resulting color for the nuclei (blue, black) cytoplasm (various shades of pink) and finally the red blood cell (red color).

Statistical analysis: Count Data are presented as mean ± SEM. Statistical analyses were performed using GraphPad Prism Demo (6.07). Values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Effects of ethanol extract of Annona muricata on change body weight in 1,2 – dimethylhydrazine induced wistar rats: The results showed that rats treated with ethanol extract of *Annona muricata (*EEA) only revealed an insignificant decrease in change in weight relative to that of control. Rats treated with DMH only showed a decrease in change in weight that is significant to that of the control group ($p < 0.05$). Co-treated DMH and EEA (DMH + EEA) groups showed significant decrease in change in weight in relation to control and DMH group. Change in weight in rats treated first with extract then DMH (EEA and DMH) showed no significant decrease relative to control, no significant increase when compared to DMH and no significant decrease when compared to EEA and no significant increase when compared to DMH + EEA group. Finally, there was a decrease in change in weight which is not significant in rats treated with DMH first then EEA (DMH and EEA) group in contrast to those in control and other groups but there was a significant increase when compared with DMH group.

Effects of Ethanol Extract of Annona muricata on Cysteine-Aspartic Proteases 3 (Caspase 3) in 1,2 – Dimethylhydrazine Induced Wistar Rats: The DMH treated group and the group treated with ethanol

extract of *Annona muricata* leaves for 12wks followed by DMH administration for another 4wks (EAA+DMH) differ significantly when compared to the normal control ($p < 0.05$). The other groups were not significantly affected by the treatment.

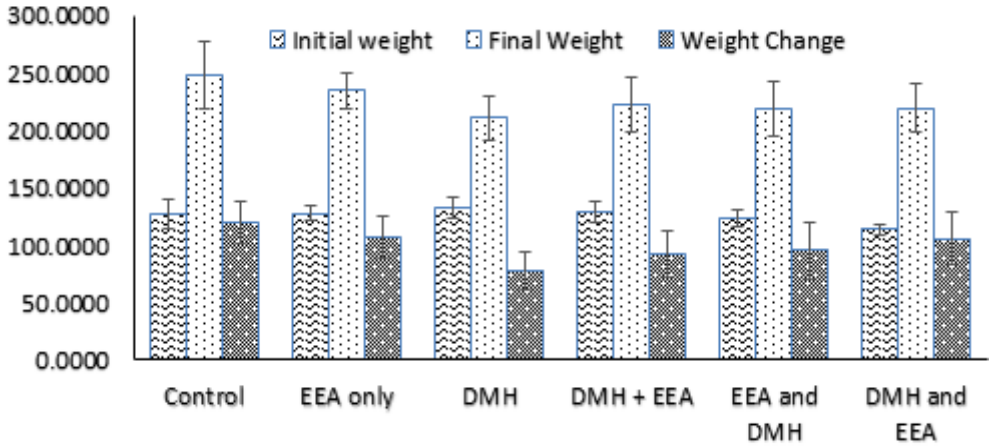

Fig 1: Effect of ethanol extract of *Annona muricata* leaves on body weight in 1. 2- dimethylhydrazine induced in rats. Data expressed as mean ± SEM (n=5). Value were said to be statistically significant from the normal control value at ($p < 0.05$).

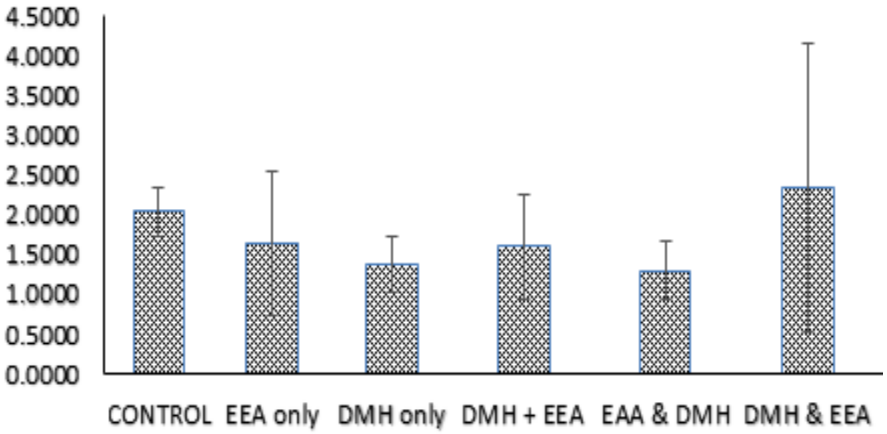

Fig 2: Effects of Ethanol Extract of Annona muricata on Cysteine-Aspartic Proteases 3 (Caspase 3) in 1,2 – Dimethylhydrazine Induced Wistar Rats. Value were said to be statistically significant from the normal control value at ($p < 0.05$).

Effect of Ethanol Extract of Annona muricata leaves on different hematological parameters: There was no significance difference between the groups that received the extract alone when compared with control ($p > 0.05$). However, some of the DMH treated groups differ significantly from the control ($p < 0.05$). These results are shown in tables 1-4. Results for lipid peroxidation and catalase: There was no significant difference between the group that received the extract alone when compared with control ($p > 0.05$) for both MDA level and catalase activity. However, some of the DMH treated groups differ significantly from the control ($p < 0.05$). These results are shown in table 5

Table 1: Effects of ethanol extract of *Annona Muricata* on some hematology indices in 1,2 – dimethylhydrazine induced wistar rats

	WBC ($x10^9$/L)	LYM (%)	MID (%)	GRAN (%)
Control	11.87 ± 2.61	72.07 ± 2.02	19.67 ± 2.42	8.27 ± 0.47
EEA	10.93 ± 1.23	71.37 ±0.68	17.20 ± 1.94	9.43 ± 2.62
DMH	17.33 ± 0.90[a]	90.40 ± 5.06[a]	15.63 ± 1.21	15.97 ± 4.75[a]
DMH + EEA	13.03 ± 1.22	75.80 ± 5.93	15.17 ± 2.75	9.03 ± 4.15
EEA and DMH	14.37 ± 1.21	74.93 ± 1.50	19.97 ± 1.73	14.10 ± 0.75[a]
DMH and EEA	11.80 ± 0.85	70.37 ± 3.86	18.23 ± 0.45	10.40 ± 3.48

All values are expressed as mean ± SEM (n = 5). Values with superscript ([a]) differ significantly from the normal control value ($p < 0.05$).
WBC = white blood cell; LYM= lymphocytes, GRAN = granulocytes.

Table 2: Effects of ethanol extract of *Annona Muricata* on some blood indices in 1,2 – dimethylhydrazine induced wistar rats

	RBC ($\times10^{12}$/L)	HB (g/dl)	HCT (%)	MCV (FL)	MCH (pg)
Control	8.43±0.33	14.97 ± 0.32	48.67 ± 2.03	57.80 ± 1.59	17.73 ± 0 .41
EEA	8.43 ± 0.18	15.00 ± 0.29	49.00 ± 1.37	58.17 ± .64	17.73 ± 0.19
DMH	9.44 ± 0.65	15.80 ± 0.90	53.00 ± 3.35	56.27 ± 0.38	16.70 ±0.21[a]
DMH + EEA	8.63±0.06	14.77 ± 0.07	49.53 ± 1.07	57.43 ± 0 .96	17.03 ± 0.12
EEA and DMH	8.31 ± 0.40	15.20 ± 0.49	51.40 ± 1.80[a]	62.17 ± 3.09	18.30 ± 0.05[a]
DMH and EEA	9.32 ± 0.35	16.60 ± $_{0.30}$a	54.17 ± 0.41	58.33 ± 1.82	17.80 ± 0.35

All values are expressed as mean ± SEM (n = 5). Values with superscript ([a]) differ significantly from the normal control value (p < 0.05). RBC = red blood cells, HB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin.

Table 3: Effects of ethanol extract of *Annona Muricata* on some blood components in 1,2 – dimethylhydrazine induced wistar rats

Group	PLT ($\times10^{11}$/L)	MPV (FL)	PDW (%)	PCT (%)	P-LCR (%)
Control	8.01 ± 1.00	7.17 ± 0.49	9.77 ± 0.92	0.58 ± 0.11	14.13 ± 3.08
EEA	7.20 ± 0.48	8.27 ± 0.81	10.85 ± 1.55	0.58 ± 0.03	23.93 ± 7.08
DMH	8.20 ± 0.52	9.67 ± 0.70[a]	13.30 ± 0.00	0.79 ± 0.05	36.90 ± 7.37[a]
DMH + EEA	9.00 ± 1.21	7.87 ± 0.75	9.85 ± 1.25	0.70 ± 0.10	20.37 ± 6.46
EEA and DMH	6.80 ± 0.93	8.53 ± 0.39	12.95 ± 0.95	0.57 ± 0.06	26.50 ± 3.86
DMH and EEA	9.34 ± 0. 68	8.57 ± 0.71	10.20 ± 0.00	0.79 ± 0.10	25.47 ± 5.96

All values are expressed as mean ± SEM (n = 5). Values with superscript ([a]) differ significantly from the normal control value (p < 0.05). PLT = platelet. MPV = mean platelet volume, PDW = platelet distribution width, PCT = procalcitonin test, P-LCR = platelet large cell ratio.

Table 4: Effects of ethanol extract of *Annona Muricata* on some blood components in 1,2 – dimethylhydrazine induced wistar rats

Group	MCHC (g/dl)	RDW-CV (%)	RDW-SD (fl)	P-LCC ($\times10^{11}$/L)
Control	30.77 ± 64	18.87 ± 0.95	29.23 ± 0.62	1.17 ± 0.37
EEA	30.5 ± 28	20.23 ± 0.78	29.23 ± 0.62	1.65 ± 0.39
DMH	29.77 19	20.17 ± 1.79	29.7 0.4	2.98 ± 0.5
DMH + EEA	29.77 ± 54	15.2 ± 0.49	29.00 ± 1.21	1.15 ± 0.64
EEA and DMH	29.57 ± 64	21.57 ± 1.27	33.07 ± 2.77	1.74 ± 0.11
DMH and EEA	30.60 ± 3.6	19.47 ± 1.24	30.13 ± 1.2	2.38 ± 0.62

All values are expressed as mean ± SEM (n = 5) all these parameters were not significantly affected by the treatments given (p > 0.05). MCHC = mean corpuscular hemoglobin concentration, RDW = red blood cell distribution width.

Table 5: Effects of ethanol extract of *Annona Muricata* on catalase and malondialdehyde in 1,2 – dimethylhydrazine induced colon and liver toxicity in wistar rats

	COLON		LIVER	
	CAT ($Kmin^{-}1$)	MDA (mol/mg protein)	CAT ($Kmin^{-}1$)	MDA (mol/mg protein)
Control	1806.28 ± 0.87	56.99 ± 0.83	1706.28 ± 0.87	45.99 ± 0.83
EEA	1687.32 ± 1.04	57.32 ± 1.04	1689.11 ± 2.74	49.11 ± 2.74
DMH	1212.46 ± 0.27[a]	78.92 ± 0.86[a]	1314.82 ± 0.53[a]	62.03 ± 2.72[a]
DMH + EEA	1447.95 ± 1.15[a]	62.95 ± 1.15	1619.61 ± 2.91[a]	51.61 ± 2.91
EEA and DMH	1310.57 ± 1.91[a]	60.57 ± 1.9[a]	1489.43 ± 2.51[a]	59.43 ± 2.5[a]
DMH and EEA	1414.03 ± 3.12[a]	54.03 ± 3.12[a]	1562.08 ± 2.66[a]	42.08 ± 2.66[a]

All values are expressed as mean ± SEM (n = 5). Values with superscript ([a]) differ significantly from the normal control value (p < 0.05)

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Histopathological examination showed that the treatment with 1,2- dimethylhydrazine, caused tumor growth in the colon and severe vascular ulceration, zonal necrosis and heavy periportal inflammatory infiltration when compared with control colon. Group treated with ethanol extract of *Annona Muricata* leaves showed normal epithelium cells further with no growth effect on the colon epithelial lining of the mucosa with no signs of cell injury. These histopathological alterations are indicated in plates 1 to 6. The carcinogenesis and development of colorectal cancer is a multistep process, characterized by progressive changes in the amount or activity of proteins that regulate the proliferation, differentiation, and cell survival, and that are mediated by genetic mechanisms (Perse and Cerar, 2011). An ordered sequence of non-random events leads to the development of colorectal cancer, with the epithelium undergoing an invasive transformation. 1,2 – Dimethylhydrazine (DMH) is a potent carcinogen that results in colon cancer in experimental rats. The ultimate carcinogenic metabolite of DMH is responsible for methylation of the DNA bases of various organs, including epithelial cells in the proliferative compartment of the colon crypts, which results in a great loss of colonic cells by apoptosis, an increase in proliferation (Ilan *et al*., 2010) and an apparent increase in mutations of colonic epithelial

cells. In this study, there was no mortality and also from the results, a significant decrease in change in body weight of the animals treated with the carcinogen (DMH) was observed. Loss of weight is one of the indices associated with cancer (Nadeem *et al*., 2021). 1,2-dimethylhydrazine-induced colon cancer is associated with significant production of oxygen free radicals leading to increased lipid peroxidation, which causes damage to cells and leading to carcinogenesis (Crosara *et al*., 2014). Imbalance between the level of MDA and Catalase can be used as an important prognostic factor in improving the survival of cancer patients. In this study the MDA levels increased significantly with the administration of 1,2 dimethyl hydrazine ($p < 0.05$).

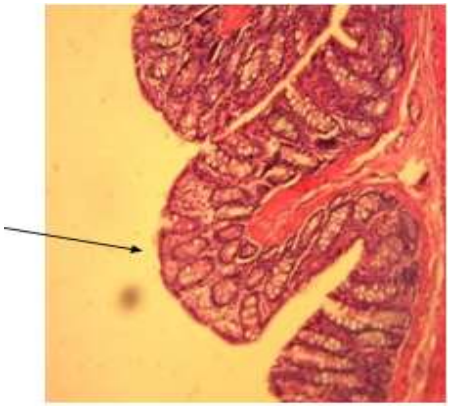

Plate 1 control: A photomicrograph of the colon shows normal epithelial lining of the mucosa, lamina propria and muscular in mucosa were preserved in control and no features of damage.

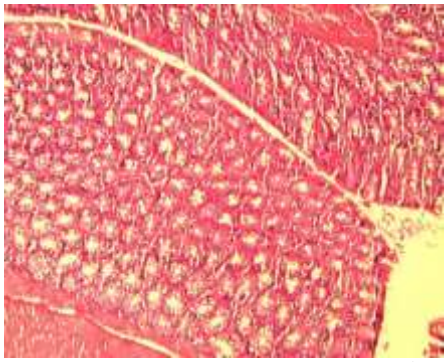

Plate 2: (EAA only): The photomicrograph section of the colon shows normal epithelial lining of the mucosa with no features of cell injury. The lamina propria and muscularis mucosa are essentially normal.

This increase in MDA levels with the administration of DMH, indicates oxidative stress caused by lipid peroxidation in the rats which can depict the presence of cancerous growth (Bakan *et al*.,2002; Eboh *et al*., 2015). MDA levels are significantly higher and antioxidant parameters, such as CAT activity, lower compared to healthy controls (Woo *et al*., 2012). Patients with colorectal adenomas have been found to have higher MDA levels as well as patients with malignant disease. Antioxidant enzymes are considered to be the first line of cellular defense against oxidative damage. It is evident from these studies that, in DMH-treated rats, the oxidant-antioxidant homeostasis was disturbed. This study showed that ethanol extract of *Annona muricata* could act as a free radical and superoxide radical scavenger, as well as preserving the activity of various antioxidant enzymes such as catalase.

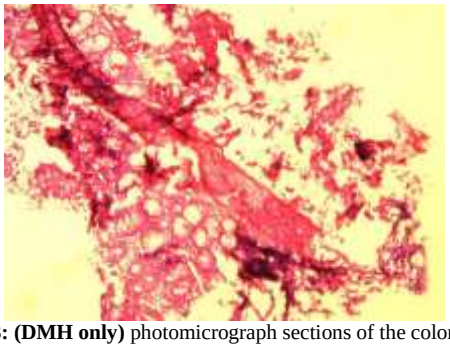

Plate 3: (DMH only) photomicrograph sections of the colon show an area of ulceration of the mucosa in which there is fragmentation of epithelial cells. The histology appears distorted.

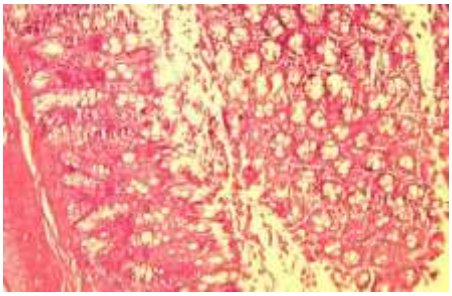

Plate 4: (DMH + EAA) Photomicrograph sections of the colon show an area of erosion of the lining epithelium of the mucosa in which there is desquamation of epithelial cells. The lamina propria and muscularis mucosa are essentially normal.

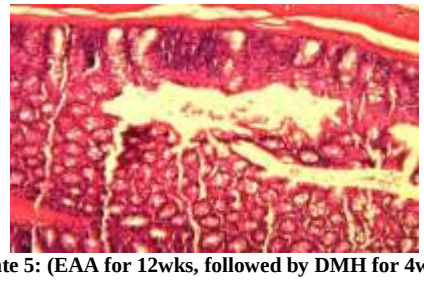

Plate 5: (EAA for 12wks, followed by DMH for 4wks) photomicrograph sections of the colon show an area of erosion of the lining epithelium of the mucosa in which there is desquamation of epithelial cells. The lamina propria and muscularis mucosa are essentially normal.

Caspases are a class of cysteine aspartic proteases that are mainly divided into two groups according to their functions in apoptosis (caspase-3/6/7/8/9) and inflammation (caspase-1/4/5/12) (MacIlwain *et al*., 2013). Caspase activity is a double-edged sword.

Although defective caspase activation and the inadequate cell death that results can promote tumorigenesis, extreme caspase activation and the excessive cell death that ensues can promote neurodegenerative conditions. Furthermore, insufficient activation of caspases involved in inflammation can lead to an increased susceptibility to infection, whereas hyperactivation of these caspases can promote inflammatory conditions (Philchenkov *et al*., 2004).

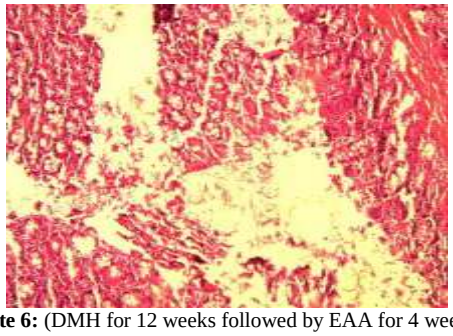

Plate 6: (DMH for 12 weeks followed by EAA for 4 weeks) photomicrograph sections of the colon show an area of erosion of the lining epithelium of the mucosa in which there is desquamation of epithelial cells. The lamina propria and muscularis mucosa are essentially normal.

Caspase3 level in DMH only and pretreatment group was low when compared to control and EEA group. This shows that EEA as treatment for DMH induced colon cancer supports the activity of Caspase3 thereby reducing the risk of colon cancer. Alterations to the Caspase 3 gene encoding the crucial executioner Caspase-3 might promote human tumorigenesis (Hosgood *et al*., 2008). Histopathological results showed that DMH caused severe inflammation, hyperproliferative cells, necrosis, ulceration and muscle layer damage in the DMH control group compared to healthy control. Treatment with ethanol leaf extract of *Annona muricata* reduces the inflammation caused by DMH and improves muscle damage recovery at a dose of 120 mg/kg compared to the DMH control. Hematological parameters generally provide information on inflammation, necrosis, various infections of visceral organs and the presence of stress factors (Abu *et al*., 2021). White blood cells play the main role in immune responses. These cells carry out the many tasks required to protect the body against disease-causing microbes and abnormal cells (Lee *et al.*, 2012; Usunobun, 2014). In the present study, DMH increased the WBCs, lymphocytes and granulocytes in DMH alone treated group compared to control and extract pretreated and post treated groups. Pretreatment with *Annona muricata* prior to toxicant administration has been found to enhance hematological parameters (Usunobun and Okolie, 2015). Pretreatment and post treatment with 120mg/kg Annona muricata significantly kept the other hematological parameters within the normal range. The major function of the RBCs is to transport hemoglobin, which in turn carries oxygen from the lungs to the tissues [Woo *et al.*, 2012]. Very low readings for RBC, hemoglobin and hematocrit can indicate anemia. Lower-than-normal hemoglobin may be due to anemia (various types), bleeding, destruction of RBCs, leukemia. *Annona muricata* is known to have wide therapeutic applications in folk medicine and scientific advancement has provided substantial evidence to support most of its medicinal claims (Olude *et al.*, 2020; Omoregie *et al*., 2020). The presence of some chemical compounds in *Annona muricata* such as tannins, flavonoids, phenols, anthraquinones, acetogenins and its positive effect on both enzymatic and non-enzymatic antioxidant has improved its potency in reducing the effect of DMH. This present in vivo study has further demonstrated the protective potential of this plant. Therefore, *Annona muricata* may be used to protect against toxic effects of DMH and other chemical agents.

Conclusion: From the findings of this result it shows that ethanol extract of *A. Muricata* leaves have both therapeutic and prophylactic effects on 1,2, dimethyl hydrazine (DMH) induced colorectal and liver cancer.

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