# GROSS HISTOLOGICAL AND HEMATOLOGICAL PROFILE OF β-CARYOPHYLLENE AND MIFEPRISTONE IN 1, 2-DIMETHYLHYDRAZINE-INDUCED RAT MODEL OF COLON CANCER

**AKINWUMI Kazeem Akinyinka**
Department of Chemical Sciences, Bells University of Technology, Ota
qaakinwunmi@yahoo.co.uk
**ADEGBOLA Oluwatosin Oluwabukola**
Department of Science Laboratory and Technology, Federal Polytechnic, Ilaro
oluwatosin.adegbola@federalpolyilaro.edu.ng

**Abstract**
*Colorectal cancer is one of the most frequently encountered malignant tumors and a major cause of cancer-related death worldwide. Eighty male Sprague Dawley rats were divided into eight groups of ten rats each and treated with 5mg/kg body weight BCP; 10mg/kg body weight BCP; 0.5mg/kg body weight MFP; and20mg/kg body weight of DMH either alone or in combination. The control rats were administered with distilled water. The BCP was orally administered daily, while oral administration of MFP was done thrice in a week throughout the twenty-week duration of the experiment. The DMH was also injected (i.m) once weekly. The impact of the treatment on body weight, hematology and gross histology were assessed following treatment. The rats treated with DMH only had declined body weight, Packed Cell Volume and Lymphocyte. In contrast, white blood cell count and neutrophils were increased in the same group. Swollen colon, cyst, were also observed in the DMH-treated rat. Co-treatment with the two doses of BCP or MFP countered and reversed gross histological and hematological alterations towards that of the control. Therefore, the result of this study suggest that β-caryophyllene and mifepristone has chemopreventive potentials for the prevention of colon cancer.*

***KEYWORDS**: Colon cancer, 1, 2-dimethylhydrazine, β-caryophylene, mifepristone, chemopreventive potential*

## Introduction

Morbidity and mortality from colon cancer are on the rise in developing countries, despite the fact that it is the third most common disease in men and the second most common cancer in women in developed nations (Bray *et al*., 2018). Colorectal cancer is strongly linked to dietary factors. People are more likely to adopt a western eating style, characterized by high fat, high protein, low carbohydrate, and low dietary fibre, as a result of rapid urbanization and widespread improvement in economic conditions. Most cases of colon cancer occur in adults over the age of 50, and the risk increases with age (Martinello *et al*., 2017). However, younger age groups are now also being affected. Male rats are more susceptible to colon cancer, hence they are often used in studies of preneoplastic and neoplastic lesions in colon carcinogenesis (Bray *et al*., 2018). Men are more likely to be diagnosed with colon cancer than women. Colorectal cancer seems to be less common in women, which may be attributable to hormonal differences. However exposure to oestrogen mitigates this risk (Karthikkumar *et al*., 2020). Colon cancer risk factors include (i) a longer than normal transit time through the intestines; (ii) a smaller than normal stool as a result of thickened luminal content; and (iii) potential interactions with various carcinogens or promoting factors already present in the intestines (Karthikkumar *et al*., 2015). Hematology abnormalities are typically associated with human cancer including colon cancer. Anemia, leukocytosis and thrombocytosis were recorded to be fundamentally higher in colorectal malignant growth patients than the non-malignant disease patients (Qiu *et al*., 2010). Cancer related leukocytosis is viewed as a significant paraneoplastic condition (Chen *et al*., 2020). This type of cell is referred to as differentiated. Undifferentiated or, due to the fact that they frequently appear to be very immature cells, primitive cancer cells, which 11 include colon cancer, are cancer cells that do not resemble healthy counterparts (Mayani *et al*., 2022).

The enzyme nitric oxide synthase (NOS) aids in the formation of reactive nitrogen species (RNS), which are primarily derived from arginine. Peroxynitrite (ONOO) is produced when NOS reacts with oxygen to form the radical nitric oxide (NO•). (Förstermann and Sessa, 2012). According to Lee *et al*. (2014), ROS-induced DNA damage and genomic instability are crucial to the onset and progression of various cancers, including colon cancer.

An alkylating agent that is frequently used to induce both benign and malignant neoplasms in rodents' colons is the organotropic colon carcinogen DMH. DMH exposure in animals results in colon tumors with pathological characteristics comparable to those of sporadic forms of human colon cancer. The verifiable background of DMH starts around 1965, Laquer (1965) investigating the neurotoxicity for seed of Cycas circinalis L. Additionally, it was discovered that the primary metabolite of aglycone cycasin (MAM), a glycoside isolated from the rough material, and a -D-glucosyloxy azoxymethane are responsible for intestinal cancers (Karthikkumar *et al*., 2020). The 1, 2- Dimethylhydrazine is used as a development control specialist in plants as well 0as fuel in rockets and jets. Procarcinogens like DMH and its metabolite, azoxymethane (AOM), require metabolic initiation to form DNA-reactive products. Since MAM is a substrate of the colon and liver's nicotinamide adenine dinucleotide (NAD)-dependent dehydrogenase, the active metabolite of MAM may be the corresponding aldehyde. After hepatic conversion to MAM and azoxymethanol, DMH is primarily removed via glucuronic acid conjugation and biliary excretion (Sohn *et al*., 2011). Azoxymethanol poisoning releases aspartate and alanine amino transferases and alkaline phosphatase, suggesting damage to liver and other organelle (Pöltl *et al*., 2023). Bacterial hydrolysis of glucuronides in the intestinal lumen yields active carcinogen. Expanded frequencies of lung, nasal depression, colon and liver growths have been seen in rodents presented to hydrazine. Restricted creature studies propose that hydrazine is discharged essentially in the pee and air exhalation (Ivanovo and Lee, 2023). β-caryophyllene sesquiterpenes are distinguished from other types of chemical compounds by their fused dimethylcyclobutane and nine-membered rings of the β-caryophyllene skeleton. Adding the β-caryophyllene framework is formed when a trans-endocyclic (4-5) double bond joins the nine-membered ring of the β-caryophyllene system (Di *et al*., 2020). A number of in vitro and in vivo experiments have demonstrated that β - caryophyllene increases antioxidant cell defense and inhibits the production of proinflammatory factors (Fidyt *et al*., 2016). β -caryophyllene was displayed to have antifungal, genoprotective, cancer prevention agent, calming, and antiproliferative properties humulene was featured for its antibacterial, antifungal, antiproliferative, and chemosensitizing activities (Loizzo *et al*., 2008). Interestingly, notwithstanding conditional signs of antifungal and antiproliferative qualities, little is known with respect to the pharmacological impacts of isocaryophyllene (Legault and Pichette, 2007). β-caryophyllene, in contrast to normal fibroblast, inhibited the proliferation of human MG-63 osteosarcoma cells (Annamalai *et al*., 2020). Non-cancerous cells like cholangiocytes, fibroblasts, and retinal ganglion cells, on the other hand, are resistant to β-caryophyllene (Chen *et al*., 2020). β-Caryophyllene killed neuroblastoma, lymphoma, glioblastoma, osteosarcoma, 42 and oral cancer cells , whereas humulene killed liver cancer cells in vitro (Chen *et al*., 2020). Mifepristone is likewise an oral kind II progesterone receptor modulator which invigorates DNA restricting and advances progesterone receptor phosphorylation (Check and Cohen, 2013). Mifepristone is a weak antiandrogen, antiglucocorticoid, and antiprogestogen. Mifepristone works better than dexamethasone or cortisol to block the progesterone and glucocorticoid receptors. Goyeneche *et al*. (2015) found that MF had antiproliferative effects on cervical, breast, endometrial, ovarian, gastric, lung, brain, and prostate-derived cancer cells.

**Materials and Methods**

Eighty adult male Sprague dawley rats weighing 160-180g were obtained. Care for the rats followed guidelines established by Guide for the Care and Use of Laboratory Animals (the Guide) and permission (FMCA/470/HREC/01/2023/12) to undertake the studies was obtained at the Federal Medical Centre, Abeokuta, Ogun State. The rats were housed in polypropylene cages with rice husk bedding and fed a standard pellet diet for a week to acclimatise them. After that, the rats were randomly divided the rats into eight groups and housed them in an incubator with a 12/12-hour light/dark cycle, 25°C temperatures, and 80% relative humidity. During the course of the experiment, all rats were provided with an abundance of water and the experimental diet containing 22% crude protein, 5% fat, 4.3% crude fibre, 1.2% calcium, 0.4%

available phosphorus, 0.56% methionine, 1.2% lysine and 300Kcal/kg metabolizable energy which was obtained from Hybrid Feeds Limited, Ibadan, Oyo state. The treatment received by the test and control rats is summarized below :1 Distilled Water 2 5mg β -carophyllene 3 10mg β -carophyllene 4 0.5mg Mifepristone 5 20mg 1,2-Dimethylhydrazine 6 5mg β -carophyllene + 20mg 1,2-Dimethylhydrazine 7 10mg β -carophyllene +20mg 1,2-Dimethylhydrazine 8 0.5mg Mifepristone + 20mg 1,2-Dimethylhydrazine. On the last day of treatment, the animals fasted overnight, and their blood was collected through ocular puncture. Following blood collection, the rats were sacrificed using cervical dislocation method. The colon was harvested from all the experimental animals, washed in saline buffer, blotted on filter paper and weighed using analytical balance. Prior to weighing, gross histological evaluation of the colon was carried out. The colon was weighed and homogenized in 5x the volume of their weight using Tris homogenate buffer (pH 7.7). The homogenate was centrifuged for 15 minutes at 15000 rpm using a cold centrifuge (Himac CR21G). The supernatant was decanted and was preserved in the freezer at $-20^0$ and later used for further analysis. The results are displayed as the mean and standard error of the mean. Intergroup differences and mean separation were achieved with one-way analysis of variance and the Duncan multiple range test respectively. Statistical significance was put at $P<0.05$.

**Results**

The weight of the DMH-treated rats decreased steadily from the tenth week to the end of the experiment, as shown in Figure 4.1, which displays the results of the weekly weight of test and control rats. Co-treatment with either of two dosages of BCP (5 or 10 mg/kg body weight) and 0.5 mg/kg body weight of MFP seemed to reverse the weight loss seen in DMH-treated group. The impact of β-caryophyllene and mifepristone on the DMH-induced changes in haematological parameters is displayed in Figure 4.2. When compared to the control group, the PCV was significantly (p0.05) reduced by 17.2% after DMH treatment, with further reductions of 11.5% and 7.8% when co-exposed to 5mg or 10mg of BCP respectively as compared to the control . The PCV in the DMH and MFP group improve to 10.8% in comparison with the control. When comparing the DMH group to the control group, the white blood cell count (WBC) increased by 39.5%. When both BCP dosages were given at the same time, they brought the white blood cell count down to almost the level of the control group. Co-exposure with MFP also reduced WBC, but to a lesser extent than the values obtained for each of the two dosages of BCP. When compared to the control group, the proportion of neutrophils in the DMH-treated group was 24.2% higher. However, the proportion of neutrophils was lowered to 15.8% when DMH was combined with any of the two dosages of BCP, while MFP lowered to 13.7%. The proportion of lymphocytes was comparable across the group, albeit the DMH-treated group had the lowest value. The gross histopathology of animals in the experimental and control groups is displayed in Figure 4.3. Macroscopic examination of DMH-treated mice revealed that their colons were transparent and included easily discernible nodules and cyst. In contrast, colons of control animals and other treatment groups were opaque and lack nodules and cysts.

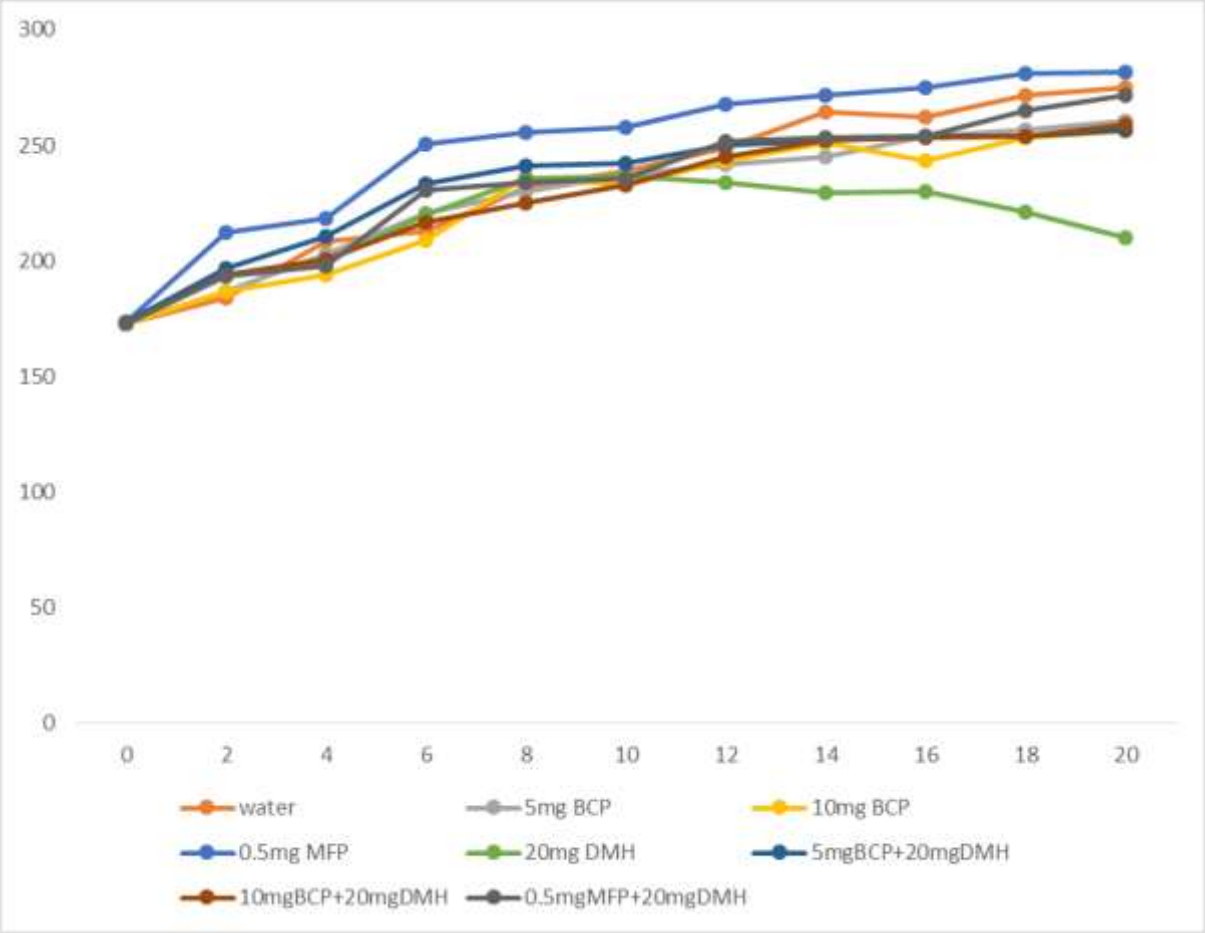


Figure 1: Body weights variation of test and control rats during the course of the treatment

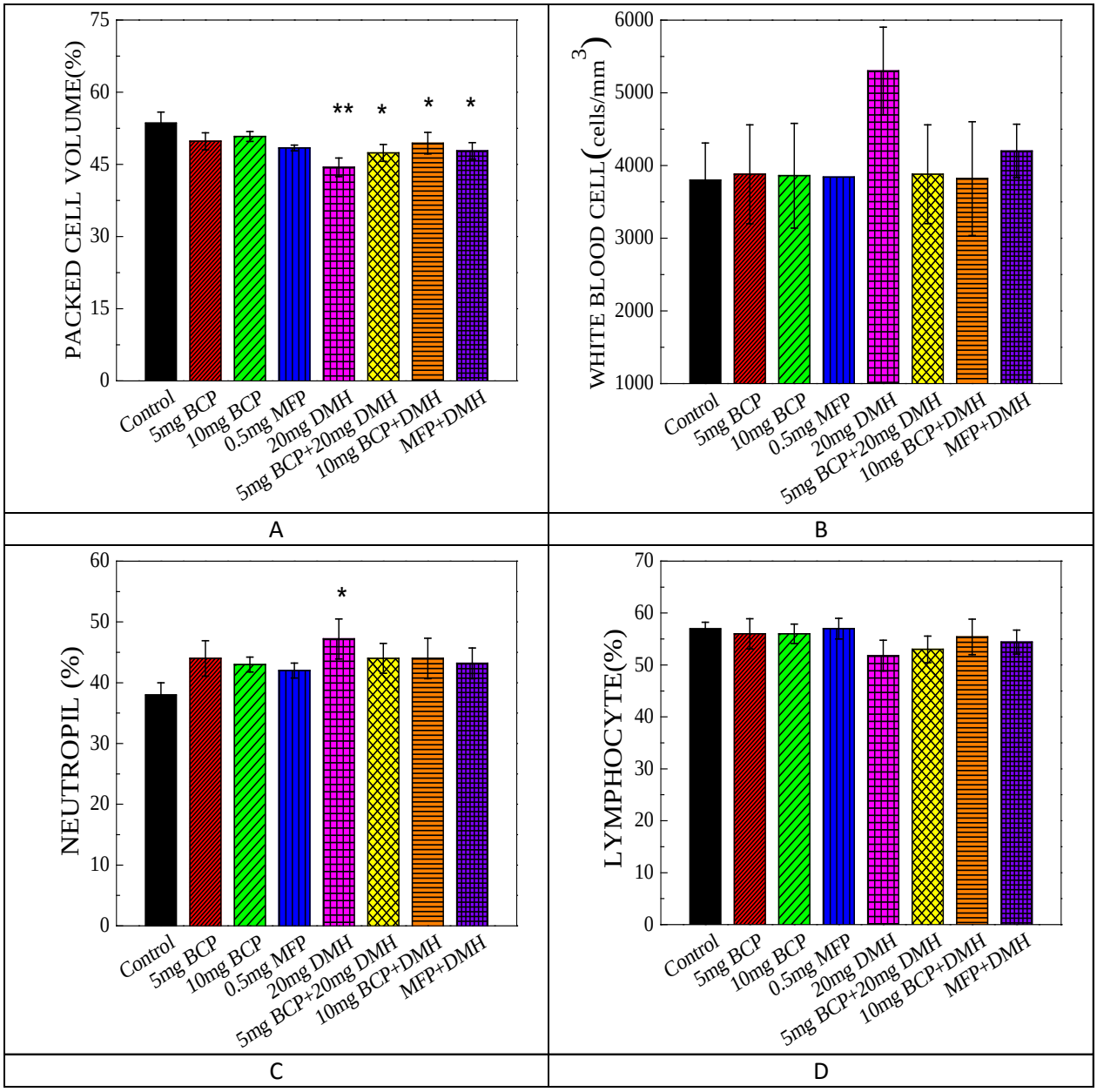


Figure 2: Effect of BCP and MFP on hematology of DMH-induced colon cancer.
* Significantly different from control, # significantly different from DMH

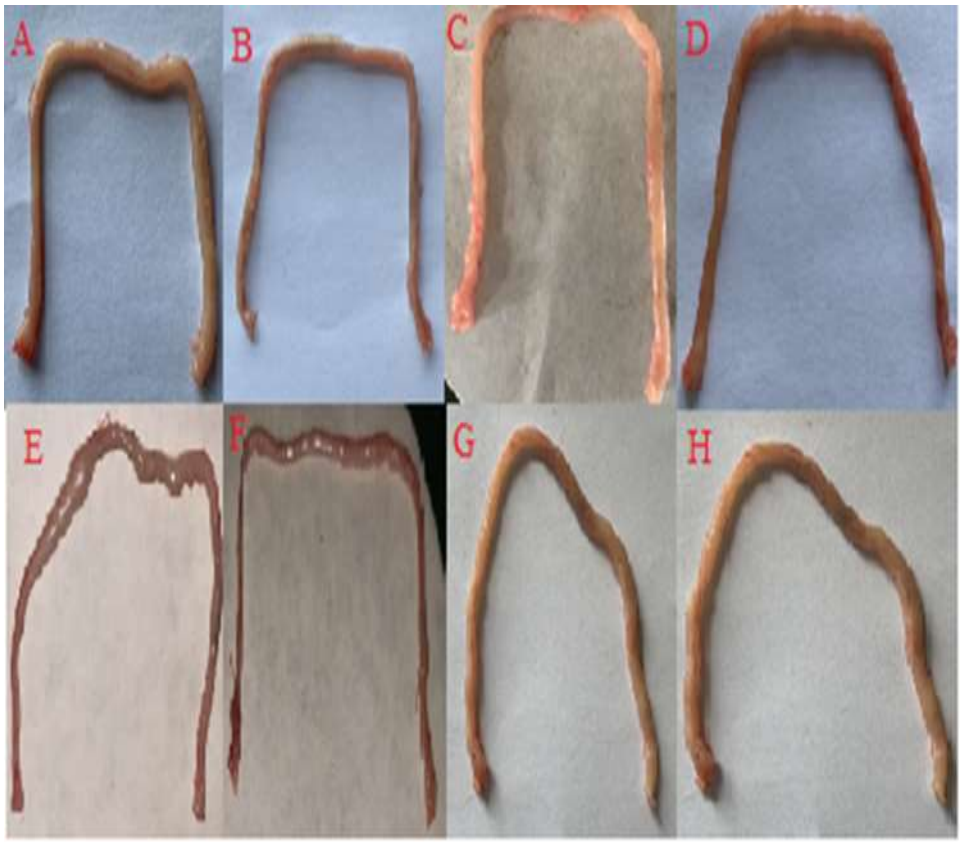


Figure 3 Gross histology of the colon in test and control animals. A- (Control group) B (BCP 5mg/kg b.w), C (BCP 10mg/kg b.w), D (MCP 0.5mg/kg), E (DMH 20mg/kg b.w,F (BCP 5mg/kg + DMH 20mg/kg), G(BCP 10mg/kg + DHM 20mg/kg) and H (MCP0.5mg/kg + 20mg/kg)

The lower body weight observed in the DMH-treated group suggests that DMH-induced colon cancer is associated with an adverse effect on animal growth. Similar results were observed by Darband *et al.* (2020) and Karthikurmar *et al*. (2015) in rodents. The progressive weight loss observed in the DMH-treated rats in this study from the tenth week may be due to tumour growth in the colon and cachexia in treated animals (Ni *et al*., 2020). Long-term administration of DMH is associated with tumour formation and cachexia in laboratory animals (Reis *et al*., 2022). Colon cancer-related cachexia is characterised by loss of body fats and lean body mass (Laws, 2022).

Moreover, colon cancer-induced weight loss is common in humans (Hardee *et al*., 2019). This may be due to reduced colon epithelium function, inflammation and lower nutrient absorption (Reis *et al.*, 2022). In contrast, the positive and beneficial effect of BCP and MFP on growth performance was demonstrated by significant improvement in weight in the co-treatment groups. Many oral dietary compounds, including antioxidants, have been reported to diminish DMH-induced weight loss (Shakib *et al*.,2019)

The presence of swollen colon with nodules and cysts in the DMH-treated group is suggestive of tumour or growth in the colon of the animals. Similar observations were recently documented in rats and mice (Sharaf *et al*., 2018). However, the absence of swollen nodules and cysts in the group co-treated with either of the two doses, BCP or MFP, may indicate their antitumor and anti-proliferative effects. The BCP and MFP can prevent tumour proliferation by inhibiting one or more stages of carcinogenesis (Steele, 2013; Check and Check, 2021). Although several studies earlier documented that DMH inflicts alterations in haematological profiles of experimental rodents. Most of the studies are unanimous on the fact DMH suppresses PCV, as obtained in this study (Mishra *et al.*, 2022; Jrah-Harzallah *et al.*, 2013; Salehi *et al*.,2022). The decline in PCV is suggestive of anaemia. Red blood cells are susceptible to attack by free

radicals released into the circulation during DMH metabolism, which usually results in oxidative stress and cell death. Thus, explaining the reduction of PCV in the DMH-treated group. In the present report, WBC was also elevated after DMH exposure, as reported in a recent study (Mishra *et al*., 2022). The WBC are mobilised in response to DMH-induced inflammation and tumours during cancer progression, which may account for elevated WBC in this study. However, the WBC differentials' alteration pattern differs from the earlier reports. For instance, Mishra *et al.* (2022) reported a drastic decrease in neutrophils and lymphocytes, while Salehi *et al*. (2022) reported an increase in neutrophils. Neutrophil permeation has been described in human sporadic premalignant colonic adenomas (Mclean *et al*., 2011). Herein, it was demonstrated that weekly administration of DMH for 20 weeks in Sprague Dawley rats resulted in a significant increase in neutrophils and a slight decrease in lymphocytes—simultaneous treatment with either of the two doses, BCP and MFP, ameliorated DMH- anaemia. Additionally, WBC and the differentials were restored near the control value. Thus, both BCP and MFP may be beneficial in protecting blood cells against anaemia and white blood cell pathologies during colon cancer proliferation.

**References**

Annamalai, V.; Kotakonda, M.; Periyannan, V (2020). JAK1/STAT3 regulatory effect of β-caryophyllene on MG-63 osteosarcoma cells via ROS-induced apoptotic mitochondrial pathway by DNA fragmentation. *J. Biochem. Mol. Toxicol*. 34, e22514

Bray F, Ferlay J, & Soerjomataram I. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer Journal of Clinical Science*, 68, 394–424.

Check JH & Check D. (2021) Mifepristone May Be the Best Single Pharmaceutical Agent for Treatment of a Variety of Advanced Cancers. Cancer Sci Res.; 4(2): 1-6

Check JH, Cohen R. (2013) The role of progesterone and the progesterone receptor in human reproduction and cancer. Exp Rev Endocrinol Metab.; 8: 469-484

Chen, H.; Hao, J.; Wen, Y.; Lv, Y.; Chen, L.; Yuan, J.; and Yang, X. (2020**)** Data of cytotoxicity, p53 and Akt downstream proteins and physiological indexes in hepatocellular carcinoma cells or HepG2-bearing nude mouse model administered by Humulene. Data Brief, 29, 105325.

Darband, S. G., Sadighparvar, S., Yousefi, B., Kaviani, M., Ghaderi-Pakdel, F., Mihanfar, A.,& Majidinia, M. (2020). Quercetin attenuated oxidative DNA damage through NRF2 signaling pathway in rats with DMH induced colon carcinogenesis. Life Sciences, 253, 117584-117592.

Di Sotto, A., Irannejad, H., Eufemi, M., Mancinelli, R., Abete, L., Mammola, C.L., Altieri, F., Mazzanti, G., & Di Giacomo, S. (2020). Potentiation of Low-Dose Doxorubicin Cytotoxicity by Affecting P-Glycoprotein through β-caryophyllene Sesquiterpenes in HepG2 Cells: An in Vitro and in Silico Study. *International Journal of Molecular Science, 21*, 6-33.

Fidyt, K., Fiedorowicz, A., Strzą dała, L., Szumny, A.(2016) β-caryophyllene and β-caryophyllene oxide-natural compounds of anticancer and analgesic properties. Cancer Med., 5, 3007–3017

Förstermann, U.; Sessa, W.C. (2012) Nitric oxide synthases: Regulation and function. *Eur. Hear. J. , 33*, 829–837.

Goyeneche AA, Telleria CM. Antiprogestins in gynecological diseases. Reproduction. 2015;149(1):R15-33.

Hardee, J. P., & Carson, J. A. (2022). Muscular contraction's therapeutic potential for cancer-induced wasting. *American Journal of Physiology-Cell Physiology*, *323*(2), C378-C384.

Ivanov, I., & Lee, V. R. (2023). Hydrazine Toxicology. In *StatPearls [Internet]*. StatPearls Publishing.

Jrah-Harzallah, H., Ben-Hadj-Khalifa, S., Maloul, A., El-Ghali, R., & Mahjoub, T. (2013). Thymoquinone effects on DMH-induced erythrocyte oxidative stress and haematological alterations during colon cancer promotion in rats. Journal of Functional Foods, 5(3), 1310-1316.

Karthikkumar V, Ramachandran V, Mariadoss A, Vijaya A, Nurulfiza M. I and Rajasekar P(2020) Biochemical and molecular aspects of 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis: a review**.***Toxicology Research,* ,**9**, 2–18.

Karthikkumar, V., Sivagami, G., Viswanathan, P., & Nalini, N. (2015). Rosmarinic acid inhibits DMH-induced cell proliferation in experimental rats. Journal Of Basic And Clinical Physiology And Pharmacology, 26(2), 185-200.

Laquer, H. L. (1965). Progress in Refrigeration Science and Technology 1963-1.

Law, M. L. (2022). Cancer cachexia: Pathophysiology and association with cancer-related pain. Frontiers in Pain Research, 3, 971295.

Lee, J.H.; Hwang, I.; Kang, Y.N.; Choi, I.J.; Kim, D.K. (2014) Genetic characteristics of mitochondrial DNA was associated with colorectal carcinogenesis and its prognosis. *PLoS ONE* , *10*, e0118612.

Legault, J.; Pichette, A. (2007) Potentiating effect of β-caryophyllene on anticancer activity of β-humulene, isocaryophyllene and paclitaxel. J. Pharm. Pharmacol., 59, 1643–1647.

Loizzo, M.R.; Tundis, R.; Menichini, F.; Saab, A.M.; Statti, G.A. (2008) Antiproliferative e_ects of essential oils and their major constituents in human renal adenocarcinoma and amelanotic melanoma cells. Cell Prolif., 41, 1002–1012.

Martinello F, Kannen V, Franco JJ (2017). Chemopreventive effects of a *Tamarindus indica* fruit extract against colon carcinogenesis depends on the dietary cholesterol levels in hamsters. *Food Chem Toxicol*; 107:261–269.

Mayani, H., Chávez-González, A., Vázquez-Santillan, K., Contreras, J., & Guzman, M. L. (2022). Cancer stem cells: Biology and therapeutic implications. *Archives of Medical Research*.

Mishra, S., Alhodieb, F. S., Barkat, M. A., Hassan, M. Z., Barkat, H. A., Ali, R.,& Alam, O. (2022). Antitumor and hepatoprotective effect of Cuscuta reflexa Roxb. in a murine model of colon cancer. Journal of Ethnopharmacology, 282, 114597.

Ni J, Zhang L (2020). Cancer Cachexia: Definition, Staging, and Emerging Treatments. Cancer Manag Res. Jul 9;12:5597-5605. doi: 10.2147/CMAR.S261585. PMID: 32753972; PMCID: PMC7358070.

Pöltl, L., Kitsera, M., Raffl, S., Schild, S., Cosic, A., Kienesberger, S.,& Zechner, E. L. (2023). Microbiota-derived genotoxin tilimycin generates colonic stem cell mutations. Cell Reports, 42(3).89-91

Qiu, M. Z., Xu, R. H., Ruan, D. Y., Li, Z. H., Luo, H. Y., Teng, K. Y., & Jiang, W. Q. (2010). Incidence of anemia, leukocytosis, and thrombocytosis in patients with solid tumors in China. Tumor biology, 31, 633-641.

Reis K.E. Socca A. R., de Souza B. R., Genaro S. C., Durán N., and Fávaro W. J., (2022)"Effects of combined OncoTherad immunotherapy and probiotic supplementation on modulating the chronic inflammatory process in colorectal carcinogenesis," Tissue and Cell, vol. 75, article 101747,.

Salehi Alireza, Hosseini Seyed Mohammad, Kazemi Sohrab (2022) "Antioxidant and Anticarcinogenic Potentials of Propolis for Dimethylhydrazine-Induced Colorectal Cancer in Wistar Rats", BioMed Research International, vol., Article ID 8497562, 3-4.

Shakib Khoob, M., Hosseini, S. M., & Kazemi, S. (2022). In vitro and in vivo antioxidant and anticancer potentials of royal jelly for dimethylhydrazine-induced colorectal cancer in wistar rats. *Oxidative Medicine and Cellular Longevity*, *202*.

Sohn, O. S., Fiala, E. S., Requeijo, S. P., Weisburger, J. H., & Gonzalez, F. J. (2011). Differential effects of CYP2E1 status on the metabolic activation of the colon carcinogens azoxymethane and methylazoxymethanol. *Cancer research*, *61*(23), 8435-8440.

Steele, V.E. (2013) Current mechanistic approaches to the chemoprevention of cancer. J. Biochem. Mol. Biol., 36, 78–81.