

"Evaluation of Fresh and Fermented Beet Root as Therapeutic Agents in Experimental Colorectal Cancer in Rats"

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Abstract

Beet Root (*Beta vulgaris* L.) Juice having a place to the Chenopodiaceae family, is high in dietary fibers, minerals (manganese, magnesium, potassium, sodium, phosphorus, iron, zinc, copper, boron, silica and selenium), B complex-vitamins. Cancer is a disease differentiated by the improper division and permanence of abnormal cells. When this exists in the colon or rectum, it is known as colorectal cancer. the present study was directed to explore the potential anticancer effect of fresh and fermented beet root juice on rats with colon cancer. Sixty male albino rats were divided into six groups, of eight rats each. Group 1, the negative control group, was fed on the basal diet alone. Groups 2, Positive control group, rats were subcutaneously injected once a week with 35 mg/kg b. wt. of DMH during the experimental period (12 weeks) Groups 3,4 Rats were injected once weekly with 35 mg/kg b. wt. of DMH and were orally received 3 and 5 ml of fresh beet root juice during the experimental period. Groups 5,6 Rats were injected once weekly with 35 mg/kg b. wt. of DMH and were orally received 3 and 5 ml of Fermented beetroot juice (FBRJ) during the experimental period. respectively. while treated groups colon cancer, fed on basal diet and given orally Fresh and Fermented Beet Root had a significant decrease ($p < 0.05$) in the activities of AST, ALT and ALP enzymes, and serum levels of AFP, TNF- α and NF- κ β and CEA and CA, urea, creatinine, and uric acid and MDA, and increased in serum levels of total protein and albumin, activities of antioxidant enzymes (CAT, GSH, and GPx), compared to untreated compared to group (2) rats with induced-colon cancer (positive control). Histopathological examination also showed an obvious improvement in the colon sections of the control colonic Groups 3,4,5,6 Rats were given orally received 3 and 5 ml of fresh and Fermented of beetroot juice (FBRJ) Finally, the existing study illustrated that beetroot juice could improve AST, ALT and ALP enzymes, and serum levels of AFP, TNF- α and NF- κ β and CEA and CA, urea, creatinine, and uric acid and MDA, and serum levels of antioxidant enzymes (CAT, GSH, and GPx).

Keywords: Beet Root - DMH - Antioxidant - Colon cancer.

1-Introduction

Cancer is a disease differentiated by the improper division and permanence of abnormal cells. When this exists in the colon or rectum, it is known as colorectal cancer (CRC) (**American Cancer Society, 2017**). colorectal cancer (CRC) is one of the most current cancers influencing the gastrointestinal tract (GI) and representing nearly two-thirds of all cancers presented in developed countries and is the second leading cause of cancer-related deaths. In addition, it is the third detected cancer in males and the second in females (**Siegel et al., 2017**). The common risk factors for the incidence of colon cancer include age, gender, genetic factors and inflammation resulting from bowel diseases as well as lifestyle and procarcinogens substances given in the food supply chain and environmental pollutants (**Benson, 2007**). Other risk factors include type 2 diabetes (**Tsilidis et al., 2015**), excessive body weight (**Ma et al., 2013**), smoking (**Secretan et al., 2009**) and moderate and heavy alcohol consumption (**Ferrari et al., 2007**).

Beetroot (*Beta vulgaris* L.) having a place to the Chenopodiaceae family, is high in dietary fibers, minerals (manganese, magnesium, potassium, sodium, phosphorus, iron, zinc, copper, boron, silica and selenium), B complex-vitamins (B1, B2, B3, B5, B6, folate, B12) and also have 50% of phenolic compounds, including betalains, carotenoids, phenols as well as complex carbohydrates, and inorganic nitrate (**Ceclu and Oana-Viorela 2020 and El-Beltagi et al., 2022**). Also, this vegetable has effective antioxidant activity via different bioactive compounds such as triterpenes, sesquiterpenoids, carotenoids, coumarins, flavonoids (tiliroside, astragalín, rhamnócitrín, rhamnétín, kaempferol), phenolic compound, glycine betaines, beta-cyanlins, saponins, and betalains . Betalains are water-soluble nitrogenous pigments that exist in most plants of the order Caryophyllales, and red beetroot is the rich source of this pigments due to the presence of betalains composed of red pigments (betacyanins) and yellow pigments (betaxanthins). Numerous studies demonstrated that carotenoids in beetroot like lycopene, β -carotene, and lutein have significant anticancer and chemotherapeutic activity against different cancers (**Gong et al., 2018; Langi et al., 2018 and Lechner and Stoner, 2019**).

Fermentation has been used by humans for many hundreds of years. In ancient times, Hippocrates noted that “bad digestion is the root of all evil”, and the benefits of fermented foods were known long before Louis Pasteur discovered lactic acid-producing bacteria. The earliest reports of fermented foods date back to 4000–3000 years B.C. and involve mushrooms and soybeans (**Gasbarrini, 2016**). Fermentation is an inexpensive process requiring little

energy to preserve foods, enriching them with newly formed bioactive compounds and improving organoleptic properties, digestibility, and assimilability (Samtiya, 2021).

For this reason, more attention has been paid to fermented root beet juice as a natural source of antioxidants as an endeavor to protect the human body from oxidative stress and hinder the progress of many chronic diseases, especially colon cancer.

2- Aim of the Study

This study was directed to explore the potential anticancer effect of fresh and fermented beet root juice on rats with colon cancer.

3- Materials and Methods

3.1. Materials

3.1.1 Fresh Beet root: were purchased from vegetable local market and will be identification in National Center for Agricultural Research, Cairo, Egypt.

3.1.2. Components and Preparation of Basal Diet:

The components of purified diets AIN M-93 (basal diet) as approved by Reeves *et al.*, (1993) were purchased from El-Gomhoriya Co., for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt. Corn starch was obtained from the Egyptian Starch and Glucose Manufacturing Co SAE, Cairo, Egypt. Sucrose and soybean oil and were purchased from a local market. All ingredients were formulated to meet the recommended dietary allowances for maintenance rats.

3.1.3. Rats

In the present study, fifty male albino rats 6-8 weeks old and weighed 195 ± 10 g were used. Rats were housed in the experimental animal laboratory at the Faculty of Veterinary Medicine, Cairo University. Rats were kept in metal wire cages in a standard environment of the light/dark cycle, temperature and humidity during the experimental period (12 weeks). All rats were fed freely on the basal diet and water was supplied *ad libitum*. The rats were left for one week for acclimatization prior to the start of the experiment.

3.1.4. Drugs and Chemicals:

A carcinogen 1, 2-Dimethylhydrazine (DMH) is a compound used experimentally to induce colon cancer in different animal models. It were purchased from Sigma Chemicals Co., Cairo, branch, Egypt. Kits for

biochemical analysis were purchased from the Gamma Trade Co., for Pharmaceutical and Chemical, Dokki, Cairo - Egypt.

3.2. Methods

3.2.1. Preparation of fermented beet root:

Beet root were collected and washed. Then they are sliced and dried . They are then pressed tightly into bamboo baskets lined with two to three layers. The tops of the baskets are then covered with Beet root plant leaves and fermented naturally at room temperature (15°C–25°C) for 25–30 days. *L. plantarum*, *L. brevis*, *Lactococcus lactis*, *Enterococcus faecium*, and *Pediococcus pentosaceus*, yeasts *Candida* spp., were LAB isolated from beet root (Tamang et al., 2007).

3.2.2. Induction of Colon Cancer:

A carcinogen 1, 2-Dimethylhydrazine (DMH) were freshly dissolved in 1 mMol EDTA/ 0.9% NaCl solution to confirm the complete stability of the solution prior to use. Colon cancer was induced by subcutaneous injection once a week with DMH at a dose of 35 mg/kg body weight for 12 weeks accorded to Moharib (2016).

3.2.3. Grouping of Rats:

After the acclimation period (one week), rats were divided into six groups (10 rats each) and grouped as follows:

Group 1: Negative control group, rats were kept as untreated group and subcutaneously were injected with 0.1 ml of the saline solution once weekly during the experimental period.

Group 2: Positive control group, rats were subcutaneously injected once a week with 35 mg/kg b. wt. of DMH during the experimental period (12 weeks).

Group 3: Rats were injected once weekly with 35 mg/kg b. wt. of DMH and were orally received 3ml of fresh beet root juice during the experimental period.

Group 4: Rats were injected once weekly with 35 mg/kg b. wt. of DMH and will be orally received 5ml of fresh beetroot juice during the experimental period (Klewicka et al., 2015).

Group 5: Rats were injected once weekly with 35 mg/kg b. wt. of DMH and were orally received 3ml of Fermented beetroot juice (FBRJ) during the experimental period.

Group 6: Rats were injected once weekly with 35 mg/kg b. wt. of DMH and were orally received 5ml of Fermented beetroot juice (FBRJ) during the experimental period.

3.2.5. Blood Collection and Biochemical Assay:

At the end of the experimental period (12 weeks), all rats were fasted overnight (12 hr.), anaesthetized with diethyl ether and scarified. Blood samples will be taken from the portal vein and divided into two parts. The first part of the blood sample were collected in anti-coagulated Eppendorf's tubes with ethylenediamine tetra acetic acid (EDTA) and used for the assay of differential leukocyte count. All samples were treated and spotted within 3 hrs. of the collected and analyzed within 12hr after smearing. The total number of leukocytes, lymphocytes and monocytes were estimated used a standard clinical hematology laboratory procedure as described by **Ruberto *et al.*, (2002)**.

The second taken part of the blood were collected in dry clean glass centrifuge tubes and allowed to coagulate at room temperature for serum separation. Serum were centrifuged at 3000 rpm for 15 min and taken by automatic micropipettes into clean dry sterile Eppendorf's tubes then preserved at -20°C till used for biochemical analysis of the following parameters:

3.2.5.1. Estimation of colon cancer inflammation marker (TNF- α and NF- κ β):

Tumor necrosis factor- alpha (TNF- α) as a pro-inflammatory cytokine and nuclear factor-kappa beta (NF- κ β) as a transcription factor were determined accorded to the described methods by **Cole (2009); Heemann *et al.*, (2012)** and **Adams (2009)**, respectively using ELISA kits (Glory Science Company, Taiwan).

3.2.5.2. Estimation of colon cancer tumor marker:

Carcinoembryonic antigen (CEA) and CA 9 19 as a tumor colon cancer marker.

3.2.5.3. Estimation of Serum Activities of AST, ALT and ALP Enzymes:

Serum activities of liver aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes were determined calorimetrically used a spectrophotometer adjusted at 505 nm as described by **Young (1997)**, while the activity of alkaline phosphatase (ALP) enzyme was determined used spectrophotometer DU 7400 adjusted at 510 nm accorded to the described method by **Roy (1970)**.

3.2.5.4. Estimation of serum level cancer marker (AFP):

Serum levels of alpha fetoprotein (AFP) as a traditional tumor biomarkers was determined accorded to the described methods by **Cole (2009); Heemann *et al.*, (2012)** and **Adams (2009)**, respectively using ELISA kits (Glory Science Company, Taiwan).

3.2.5.5. Estimation of Blood Urea, Uric Acid and Creatinine Levels:

Blood urea nitrogen (UN) concentration was assayed used Bio Merieux kits accorded to the method described by **Waiker and Bonventre (2008)**. Uric acid (UA) level were determined by an enzymatic colorimetrically accorded to the method of **Tietz (1995)**. Serum creatinine (Cr) concentration were assayed used a colorimetric kinetic method as described by **Young (2000)**.

3.2.5.6. Estimation of Lipid Peroxidation and Antioxidant Enzymes:

Malondialdehyde (MDA) is an aldehyde reflected to be the last compound and the most important marker for monitoring lipid peroxidation and assayed in the serum accorded to the method described by **Mihara and Uchiyama (1978)**. Serum activity of glutathione peroxidase (GPx) enzyme were assayed by enzyme-linked immunosorbent assay as described by **Mannervik (1985)**. Activities of catalase (CAT) and glutathione (GSH) enzymes were assayed spectrophotometrically accorded to methods described by **Lartillot *et al.*, (1988)** and **Smith (1985)**, respectively.

3.2.6. Histopathological Examination:

Colon specimens of all rats were removed and cleaned by rinsing with ice-cold isotonic saline to remove any foreign substances, clots and other blood cells. Then, the specimens were maintained in neutral buffered formalin solution (10%), cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Heamatoxylin and Eosin (H and E) stain for histopathological examination accorded to the technique described by **Bancroft and Gamble (2002)**.

3.2.7. Statistical Analysis:

Statistical Analysis: All obtained data were presented as mean \pm standard Error (SE). Analysis of Variance (Prism) test was used for determining the significances among different groups according to **Motulsky, (2007)**. All differences were considering significant if P-values at $P < 0.0$ (**Motulsky, 2007**).

4- Results and Discussions

4.1. Effect of Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice on tissue Levels of TNF- α and NF- κ β and serum level of AFP on rats with induced-colon cancer.

The observed results show The effects of Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice on at levels 3ml and 5 ml, Serum Levels of AFP, TNF- α and NF- κ β on rats with induced-colon cancer. in group (1) as normal rats (negative control), group (2) rats with induced-colon cancer (positive control) and treated groups colon cancer, fed on basal diet and given orally Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice. represents in Table (1) group (2) rats with induced-colon cancer (positive control) had a very highly significant increase ($P < 0.05$) in the mean values of the activity of AFP (3.15 ± 0.06 ng/ml), TNF- α (210.8 ± 3.2 pg/mg protein) and NF- κ β (4.13 ± 0.09 ng/mg protein) as comport to group (1) as normal rats (negative control). While treated groups colon cancer, fed on basal diet and given orally Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice. at levels 3, and 5 ml had a significant decrease ($P < 0.05$) in the mean values of AFP (2.70 ± 0.07 , 2.41 ± 0.02 , 2.22 ± 0.02 and 1.94 ± 0.05 ng/ml) and TNF- α (189.5 ± 2.8 , 168.3 ± 2.1 , 142.6 ± 1.9 and 116.4 ± 2.3 pg/mg protein) and NF- κ β (3.61 ± 0.08 , 2.95 ± 0.10 , 2.32 ± 0.07 and 1.90 ± 0 ng/mg protein) respectively as compared to group (2) rats with induced-colon cancer (positive control).

Natural chemicals as protective or rapeutic agents have gotten a lot of interest in recent years all around globe. Recent research has revealed that red beetroot and its active components betalains (also betanin) offer a range of health advantages, including ant oxidative, ant inflammation, anticancer, blood pressure and cholesterol lowering, as well as antidiabetic and anti-obesity properties. Betanin is a betalain glycosidic pigment that is a key component of red beetroot and is used as a food ingredient (Varshney and Mishra, 2022).

Supporting to this finding, previous study recorded that the anti-inflammatory effects of betalains and beetroot extract appear to be mediated by interfering with the NF- κ B signalling cascade (El Gamal *et al.*, 2014). The transcription factor NF- κ B promotes immunity directly by activating gene targets that up-regulates the inflammatory molecules such as chemokines and cytokines that stimulate the phagocytic cells (Baker *et al.*, 2011). In an animal model study to investigate the protective effect of beetroot extract on gentamicin-induced nephrotoxicity, up-regulation of nuclear expression of NF- κ B (p65), production of TNF- α and interleukin-16 (IL-6), myeloperoxidase activity, and the nitric oxide level were significantly down regulated upon beetroot supplementation (TAN and Hamid 2021).

AFP was the first to reveal that AFP could upregulate MACC1 activity and thus promote GC proliferation, migration, and invasion. Numerous previous studies reported that high expression levels of AFP and MACC1 are significantly associated with higher metastasis and poor prognosis in GC, respectively (**Liu *et al.*,2020**). The findings from the present study demonstrated that AFP could enhance tumor progression rather than acting as a tumor marker. This may help illustrating the aggressive behaviors of AFP-GC and common GC in patients with high AFP serum levels (**Mao *et al.*,2022**).

Serum AFP level is a prognostic factor for overall survival and treatment response in patients with AFP-GC (**Wang *et al.*,2020**). In common GC, a higher serum AFP level is also an independent factor of poor prognosis (**Chen *et al.*,2015**). However, the underlying molecular mechanism of AFP in GC progression remains unclear. A previous study reported that activation of the Wnt signaling pathway is responsible for stimulation of cell proliferation and aggressiveness enhanced by AFP in AFP-overexpressed GC cells (**Chen *et al.*,2019**). The findings from the present study demonstrated the enhancement of GC progression by AFP via upregulation of MACC1. To further confirmation this results, the correlation between AFP and MACC1 expression levels should be evaluated in human GC tissues. Additional research is therefore needed to determine the molecular mechanism underlying AFP-MACC1 regulation on GC progression (**Mao *et al.*,2022**) .

Administration of DMH induced colon cancer in rats exhibited significant increase in TNF-alpha colon tissues (Table2). The relative expression of TNF- α mRNA in colorectal cancer was significantly higher than that present in adjacent normal colorectal tissue (**Al Obeed *et al.*,2014**) .Due to metabolism of DMH, the production of excess ROS ends in the activation of TNF- α by p65-NF- κ B pathway. Enhanced level of TNF- α could damage function and transcription of the affected proteins and progression of epithelial cell transformation to invasive cancer in colonic mucosa through initiating the inflammation and progression of cancer (**Anna *et al.*, 2015 and Garza-Trevino *et al.*, 2015**). On the other hand administration of vitamin C(Protection and Treated) groups revealed that, a significant decrease in the levels of TNF- α when compared with DMH untreated group .The results are in agreement with (**Mikirova *et al.*,2013**) who reported that,TNF-alpha decreased significantly in the presence of Ascorbic acid (vitamin C, ascorbate) due to dehydroascorbic acid (DHA) inhibited cellular NF- κ B production. Moreover, administration of vitamin C led to reduction of TNF-alpha induced endothelial cell apoptosis due to this effect was mediated by suppression of the mitochondria initiated apoptotic pathway (**Toth *et al.*, 2002**) .

Table 1: Effect of Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice on tissue Levels of TNF- α and NF- κ β and serum level of AFP on rats with induced-colon cancer.

| Parameters Groups | | Parameter as Mean \pm SD | | |
|---------------------------|-----|----------------------------|-------------------------------|--------------------------------------|
| | | AFP (ng/ml) | TNF- α (pg/mg protein) | NF- κ β (ng/mg protein) |
| Negative control group | | 1.15 \pm 0.02 | 158.2 \pm 4.5 | 0.87 \pm 0.02 |
| Positive control group | | 3.15 \pm 0.06*** | 210.8 \pm 3.2*** | 4.13 \pm 0.09*** |
| Fresh beet root juice | 3ml | 2.70 \pm 0.07NS | 189.5 \pm 2.8NS | 3.61 \pm 0.08NS |
| | 5ml | 2.41 \pm 0.02* | 168.3 \pm 2.1* | 2.95 \pm 0.10* |
| Fermented beet root juice | 3ml | 2.22 \pm 0.02** | 142.6 \pm 1.9** | 2.32 \pm 0.07** |
| | 5ml | 1.94 \pm 0.05*** | 116.4 \pm 2.3*** | 1.90 \pm 0.05*** |

- Results are expressed as mean \pm SD
- Means with different superscript stars in the column are significantly different at significant * $p > 0.05$ * highly significant ($p < 0.01$); ** very highly significant ($p < 0.001$) and Ns. none significant as compared to group 2 positive group.
- AFP (Alpha fetoprotein) , TNF- α (Tumor necrosis factor- alpha) and NF- κ β (Nuclear factor-kappa beta).

4.2. Effect of Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice on CEA and CA on rats with induced-colon cancer.

The present work recorded that the effects of Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice on at levels 3ml and 5 ml, on CEA and CA on rats with induced-colon cancer. in group (1) as normal rats (negative control), group (2) rats with induced-colon cancer (positive control) and treated groups colon cancer, fed on basal diet and given orally Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice. represents in Table (2) group (2) rats with induced-colon cancer (positive control) had a very highly significant increase ($P < 0.05$) in the mean values of CEA (5.34 ± 0.07 ng/mg protein) and CA (1.17 ± 0.01 ng/mg protein) as comport to group (1) as normal rats (negative control). While treated groups colon cancer, fed on basal diet and given orally Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice. at levels 3, and 5 ml had significant decrease ($P < 0.05$) in the mean values of the activity of CEA (4.24 ± 0.11 , 3.48 ± 0.09 , 2.84 ± 0.08 and 2.35 ± 0.06 ng/mg protein) CA and (0.92 ± 0.03 , 0.82 ± 0.01 , 0.62 ± 0.02 and 0.51 ± 0.02 ng/mg protein) respectively as compared to group (2) rats with induced-colon cancer (positive control).

Betacyanin components, including betanin and betanin, possess anti-inflammatory and anti-apoptotic properties. BVEE treatment is believed to improve the extent of structural damage and reduce inflammatory infiltration in renal tubules by reducing oxidative stress, inflammation, and apoptosis in the kidney (**Mirmiran *et al.*, 2020**).

Red beet extracts were rich in flavonoids and saponins, which are known to have strong antioxidant potential and free radical scavenging properties (**Sacan and Yanardag, 2010; Al-Dosari *et al.*, 2011**).

Fermentation increases the usefulness of beetroot juice by enriching it with lactic acid bacteria metabolites, extending the range of its biological activity. It was shown that fermented beetroot juice has potent antioxidant, antihypertensive, hepatoprotective, anticancer, anti-microbial, antidepressant, hypocholesterolemic, immunomodulatory, anti-inflammatory, and probiotic properties (**Kazmierczak *et al.*, 2014; Kumari *et al.*, 2022**).

In the fermented «Beetroot drink», the number of beta-lains remained unchanged for 14 days of storage. By the literature, the fermentation process can have a preservative effect on beet juice pigments (**Czyżowska *et al.*, 2006**).

Also reported same finding a significant increase in serum AFP, CEA and CA19.9 levels were observed in DMH group when compared to normal control. The results are in agreement with Umesalma and Sudhandiran, (2010) who reported that, elevation in serum CEA concentration was observed in DMH-induced colon cancer in rats, 1, 2 di methyl hydrazine, a potent carcinogen administered, induced reactive oxygen species (ROS) damage to colon that causes instability of colon cell metabolism, which leads to different changes in tumor markers (CEA and AFP) are representatives of colon function. In addition to, serum CEA was elevated in 20% of patients at primary diagnosis of colon cancer and in 46.6% of patients at reappearance (**Chang *et al.*, 2012**). Both elevated serum CEA and CA 19-9 levels were associated with the presence CRC. Elevated serum CEA and CA 19-9 levels were significantly correlated with larger lesion size and multiplicity of adenomas (**Kim *et al.*, 2017**). Treatment of vitamin C to DMH-induced colon cancer in rats significantly reduced elevated serum AFP & CEA and CA 19-9 levels when compared with DMH non-treated group. Serum levels of CEA decreased in colon cancer patients after the post treatments of high dose intravenous ascorbic acid (**Mikirova *et al.*, 2012**). Further more, the level of AFP was significantly decreased when pretreated and post-treatment by vitamin C in albino rats (**Ahmed *et al.*, 2008**).

Table 2 : Effect of Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice on CEA and CA on rats with induced-colon cancer.

| Parameters Groups | | Parameter as Mean \pm SD | |
|---------------------------|-----|----------------------------|---------------------|
| | | CEA ng/mg protein | CA ng/mg protein |
| Negative control group | | 0.82 \pm 0.03 | 0.16 \pm 0.01 |
| Positive control group | | 5.34 \pm 0.07*** | 1.17 \pm 0.01*** |
| Fresh beet root juice | 3ml | 4.24 \pm 0.11NS | 0.92 \pm 0.03NS |
| | 5ml | 3.48 \pm 0.09* | 0.82 \pm 0.01* |
| Fermented beet root juice | 3ml | 2.84 \pm 0.08** | 0.62 \pm 0.02** |
| | 5ml | 2.35 \pm 0.07*** | 0.51 \pm 0.02*** |

- Results are expressed as mean \pm SD
- Means with different superscript stars in the column are significantly different at significant * $p > 0.05$ * highly significant ($p < 0.01$); ** very highly significant ($p < 0.001$) and Ns. none significant as compared to group 2 positive group.
- CEA (Carcinoembryonic antigen) and CA (Cancer Antigen).

4.3. Effect of Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice on Serum Activities of AST, ALT and ALP Enzymes on rats with induced-colon cancer.

The present study showed that the effects of Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice on at levels 3ml and 5 ml, on Serum Activities of AST, ALT and ALP Enzymes on rats with induced-colon cancer in group (1) as normal rats (negative control), group (2) rats with induced-colon cancer (positive control) and treated groups colon cancer, fed on basal diet and given orally Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice. represents in Table (3) group (2) rats with induced-colon cancer (positive control) had a very highly significant increase ($P < 0.05$) in the mean values of the activity of AST (199.32 \pm 0.05 U/L), ALT (222.12 \pm 0.17 U/L) and ALP (9.42 \pm 0.11 μ mol/ml) as comport to group (1) as normal rats (negative control). While treated groups colon cancer, fed on basal diet and given orally Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice. at levels 3, and 5 ml had a significant decrease ($P < 0.05$) in the mean values of AST (154.11 \pm 0.03, 133.24 \pm 0.12, 126.16 \pm 0.15 and 71.46 \pm 0.18 U/L) and ALT (182.46 \pm 0.14, 151.37 \pm 0.09, 146.42 \pm 0.02 and 88.17 \pm 0.18 U/L) and ALP (7.22 \pm 0.03, 6.36 \pm 0.22, 5.99 \pm 0.47 and 4.18 \pm 0.22 μ mol/ml) respectively as compared to group (2) rats with induced-colon cancer (positive control).

dietary nitrate, which can increase the production of nitric oxide in the body. Nitric oxide has been shown to have beneficial effects on the liver, including reducing inflammation and improving blood flow (**Milton-Laskibar et al, 2021**).

Effects of Beetroot juice on the level of liver enzymes, we found that consumption of 250 mL of red beetroot juice for 12 weeks, parallel to the Mediterranean diet, had a significant beneficial impact on some liver enzymes ALT and ALP (**Fateh et al, 2023**).

Supporting to this finding, a previous study observed that the Beta vulgaris, alongside the standard treatment of NAFLD, could have positive effects on the biochemical markers of patients with NAFLD. Integration of Beta vulgaris in the treatment regimen of NAFLD patients significantly decreased AST and ALP as the main biomarkers of hepatic disease, compared to the standard treatment. Since elevated AST is associated with higher grades of fibrosis among NAFLD patients (**Alizadeh et al.,2018**). improvement of AST is promising way to prevent the progression of liver fibrosis (**Afzali et al.,2020**).

Albrahim and Alonazi, (2020) recorded that when drink beet root to animals showed normalization of all biomarkers, while the control group exhibited sustained elevations above normal in some participants. Similar data were obtained in animal experiments, where orally administered red beetroot extract exhibited a considerable decrease in the activity of serums alanine transaminase, aspartate trans-aminase, and alkaline phosphatase and a notable increase in albumin serum and total proteins levels in rats chronically exposed to silver nanoparticles.

Beetroot drink affected lipid metabolism, normalizing elevated cholesterol and triglyceride levels in 53.0% of patients in the study group. due to the special composition of beetroot drink (content of free amino acids, including essential, organic acids, and vitamins B, PP, C, E). The hepato protective effect of the «Beetroot drink» may be due to the antioxidant properties of betalains, which protect hepatocytes from damage (**Spivak et al.,2025**).

DMH is considered to form active intermediates including azoxy methane and methyl azoxy methanol in the liver, which are moved subsequently into the colon via bile and blood. Methylazoxymethanol is decomposed to form methyl diazonium ions, which methylate cellular components. DMH also generates free radicals that induce oxidative DNA damage in the liver and colon. Damage to DNA from ROS is a consequence of oxidative stress and several oxidative DNA adducts, including 8-oxodG, have been implicated in the tumorigenic

process (Aranganathan and Nalini, 2009). To induce colorectal tumors, the potency of DMH is the reason of inducing DNA methylation (Rowlatt *et al.*, 2016), which was strongly associated with abnormal gene expression and tumorigenesis (Salehi *et al.*, 2015)

Table 3 : Effect of Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice on Serum Activities of AST, ALT and ALP Enzymes on rats with induced-colon cancer.

| Parameters Groups | | Parameter as Mean ± SD | | |
|---------------------------|-----|------------------------|----------------|---------------|
| | | AST (U/L) | ALT (U/L) | ALP (μmol/ml) |
| Negative control group | | 34.66±0.02 | 46.40±0.11 | 3.42±0.16 |
| Positive control group | | 199.32±0.05*** | 222.12±0.17*** | 9.42±0.11*** |
| Fresh beet root juice | 3ml | 154.11±0.03NS | 182.46±0.14NS | 7.22±0.03NS |
| | 5ml | 133.24±0.12* | 151.37±0.09* | 6.36±0.22* |
| Fermented beet root juice | 3ml | 126.16±0.15** | 146.42±0.02** | 5.99±0.47** |
| | 5ml | 71.46±0.18*** | 88.17±0.18*** | 4.18±0.22*** |

- Results are expressed as mean ± SD
- Means with different superscript stars in the column are significantly different at significant * p > 0.05 * highly significant (p < 0.01); ** very highly significant (p < 0.001) and Ns. none significant as compared to group 2 positive group.
- AST (Aspartate aminotransferase), ALT (Alanine aminotransferase) and ALP (Alkaline phosphatase).

4.4. Effect of Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice on Blood Urea, Creatinine and Uric Acid Levels on rats with induced-colon cancer.

On the present work, results showed that the effects of Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice on at levels 3ml and 5 ml, Serum Levels of Urea, Creatinine and Uric Acid on rats with induced-colon cancer. in group (1) as normal rats (negative control), group (2) rats with induced-colon cancer (positive control) and treated groups colon cancer, fed on basal diet and given orally Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice. represents in Table (4) group (2) rats with induced-colon cancer (positive control) had a very highly significant increase (P<0.05) in the mean values of the activity of Urea (58.77±.43 mg/dl), Creatinine (8.48±0.17 mg/dl) and Uric Acid (4.66±0.09 mg/dl) as comport to group (1) as normal rats (negative control). While treated groups colon cancer, fed on basal diet and given orally Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice. at levels 3, and 5 ml had a

significant decrease ($P < 0.05$) in the mean values of Urea (41.34 ± 0.36 , 33.11 ± 0.28 , 30.63 ± 0.21 and 26.71 ± 0.21 mg/dl) and Creatinine (6.41 ± 0.12 , 5.99 ± 0.28 , 5.24 ± 0.03 and 3.11 ± 0.17 mg/dl) and Uric Acid (3.11 ± 0.03 , 2.94 ± 0.04 , 2.14 ± 0.27 and 1.48 ± 0.81 mg/dl) respectively as compared to group (2) rats with induced-colon cancer (positive control).

In this milieu, the outcomes of the present research via dietary provision of red beetroot juice and nutraceutical beetroot drink depicted positive effects on kidney function as indicated by biochemical and histopathological indicators (**Butt *et al* , 2019**).

These results are constituent with the results of (**Mirmiran *et al* , 2020**) who investigated the beneficial contribution of either beetroot juice or nutraceutical beetroot juice in the treatment of Gentamicin-nephrotoxicity-induced rats. It was suggested that beetroot juice with prophylactic perspectives actively supported the renal system to overcome the adverse effects from the toxicant-induced damage. Therefore, consumption of beetroot- based beverages depicted positive implications by increasing the level of Catalase . Similarly, urea and creatinine content have lowered. due to the action of bioactive compounds like betacyanins and betaxanthin (**Butt *et al* , 2019**) .

Beetroot have shown to appear a significant medical values and positive health effects, its significantly decrease a damage effects on liver kidney and shown a significant decrease of lipid profile in mice subjected to dexamethasone, its unlike synthetic colorants which may start an a adverse effects in humans, the pharmacological properties of betalains- rich foods as red beetroot show their great potential as functional foods (**Bari *et al.*,2018**) .

Our novel therapy leads the postulation that natural food product with known anticancer activity as beetroot-carrot juice, when used in right combination and dose could enhance the therapeutic efficacy of the potent antileukemic drug chlorambucil, by reducing its toxic side-effects. A possible synergistic effect of consumption of beetroot-carrot juice with chlorambucil resulted in a substantial improvement in renal function by acting as a kidney cleanser and protects the patient from renal impairment. Furthermore, beetroot-carrot juice could be used as natural alternative treatment for CLL when it was daily consumed as it has an antileukemic and anticancer effect (**Shakib *et al.*,2015**).

Serum and kidneys of mice were analyzed for biomarkers of kidney health, which revealed the positive effect of red beetroot drinks. Particularly, the antioxidant enzymes in renal tissues and serum proteins were significantly

improved, whereas lipid peroxidation, nitric oxide, urea and creatinine levels were momentarily reduced in nephrotoxicity-induced rats. Furthermore, histological assessment indicated better renal portfolio in the rats treated with beet beverages. The findings suggested that red beetroot-based beverages promisingly ameliorate negative impacts of gentamicin-induced nephritic stress (Butt *et al.*,2019).

Consumption of beetroot- based beverages depicted positive implications by increasing the level of Superoxide Dismutase (as a primary antioxidant enzyme), and Catalase (involved in a detoxification procedure), while decreasing NO (with a controversial role in renal system), and oxidative stress, all as renal tissue-specific markers. Similarly, urea and creatinine content have lowered, while the protein profile of beetroot- based beverage accelerated, due to the action of bioactive compounds like betacyanins and betaxanthin (Butt *et al.*,2019) .

These results are constituent with the results of (El Gamal *et al.*,2014) who investigated the advantage of beta- vulgaris ethanol extract (BVEE) with potent antioxidant, anti-inflammatory and reno-protective properties in the treatment of GM- induced nephrotoxicity, modulation of renal dysfunction, oxidative stress, inflammation and amelioration of histological damage in rats. The administration of BVEE and GM- treatment subsequently, substantiated a significant suppressing effect on the elevation of urea, uric acid, total protein and creatinine in a dose-dependent manner.

Table 4 : Effect of Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice on Blood Urea, Uric Acid and Creatinine Levels on rats with induced-colon cancer.

| Parameters Groups | | Parameter as Mean \pm SD | | |
|---------------------------|-----|----------------------------|--------------------|--------------------|
| | | UN (mg/dl) | Cr (mg/dl) | UA (mg/dl) |
| Negative control group | | 19.48 \pm .18 | 1.74 \pm .36 | 0.55 \pm .03 |
| Positive control group | | 58.77 \pm .43*** | 8.48 \pm 0.17*** | 4.66 \pm 0.09*** |
| Fresh beet root juice | 3ml | 41.34 \pm 0.36NS | 6.41 \pm 0.12NS | 3.11 \pm .03NS |
| | 5ml | 33.11 \pm 0.28* | 5.99 \pm 0.28* | 2.94 \pm 0.04* |
| Fermented beet root juice | 3ml | 30.63 \pm 0.21** | 5.24 \pm .03** | 2.14 \pm 0.27** |
| | 5ml | 26.71 \pm 0.21*** | 3.11 \pm 0.17*** | 1.48 \pm 0.81*** |

- Results are expressed as mean \pm SD
- Means with different superscript stars in the column are significantly different at significant * $p > 0.05$ * highly significant ($p < 0.01$); ** very highly significant ($p < 0.001$) and Ns. none significant as compared to group 2 positive group.
- UN (Urea nitrogen), Cr (Creatinine) and UA (Uric acid).

4.5. Effect of Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice on Lipid Peroxidation and Antioxidant Enzymes on rats with induced-colon cancer.

In the present study the effects of Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice on at levels 3ml and 5 ml , on lipid peroxidation as indicated by serum MDA level and activity of CAT, GSH and GPx in group (1) as normal rats (negative control) , group (2) rats with induced-colon cancer (positive control) and treated groups colon cancer, fed on basal diet and given orally Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice. represents in Table (5) group (2) rats with induced-colon cancer (positive control) had a very highly significant increase ($P<0.05$) in the mean values of MDA (4.485 ± 0.335 n.mol/mg protein) as compared to group (1) as normal rats (negative control). While treated groups colon cancer, fed on basal diet and given orally Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice. at levels 3, and 5 ml had significant decrease ($P<0.05$) in the mean values of the activity of MDA (3.635 ± 0.125 , 1.935 ± 0.175 , 0.830 ± 0.010 and 0.800 ± 0.050 n.mol/mg protein) respectively as compared to group (2) rats with induced-colon cancer (positive control).

group (2) rats with induced-colon cancer (positive control) had a very highly significant decrease ($P<0.05$) in the mean values of the activity of CAT (0.755 ± 0.015 U/ gT protein) , GSH (0.940 ± 0.010 mmol/ mg protein) and GPx (1.085 ± 0.025 U/ gT protein) as compared to group (1) as normal rats (negative control) .While treated groups colon cancer, fed on basal diet and given orally Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice. at levels 3, and 5 ml had a significant increase ($P<0.05$) in the mean values of CAT (1.370 ± 0.050 , 1.895 ± 0.135 , 3.665 ± 0.085 and 3.635 ± 0.305 U/ mg protein) and GSH (1.800 ± 0.250 , 2.940 ± 0.180 , 4.295 ± 0.025 and 4.425 ± 0.185 mmol/ mg protein) and GPx (2.760 ± 0.110 , 4.010 ± 0.140 , 5.450 ± 0.130 and 5.520 ± 0.060 U/ gT protein) respectively as compared to group (2) rats with induced-colon cancer (positive control).

The observed regeneration of tissues may be due to the phytoconstituents of beetroot which can reduce lipid peroxidation, thereby stabilizing the lipid membrane of the kidney and ultimately prevent necrosis (Olumese and Oboh, 2016).

The improvement in the antioxidants activities may be due to potent antioxidant capacity of beet root juice which alleviates kidney dysfunction and structural damages through decreasing oxidative stress and inflammation in kidney tissues. The obtained results corroborate the findings of who reported

that oral administration of beet root juice for 28 days at doses of 150 and 300 mg/kg BW caused an increase in the activities of CAT, SOD and decreased MDA levels(**Raish *et al.*, 2019**) . As well as, indicated that betalains and other phenolic compounds presente in red beet decrease oxidative damage of lipids and improve antioxidant status in humans (**Netzel *et al.*, 2005**)

Similary, it has been reported that the beet root juice on antioxidant parameters in kidney of nephropathy diabetic rats are recorded in Table (5). The results indicated that positive control rats showed significantly decreases ($P \leq 0.05$) in catalase (CAT), glutathione peroxidase (GSH.px) and superoxide dismutase (SOD) activities and higher level of malonaldehyde (MDA) when compared to normal control rats. The increase in MDA levels and the decrease in CAT, GSH.px and SOD activities may be related to the action of oxidative stress resulting from hyperglycemia and gentamicin (**El Sheikh and Othman 2019**)

Moreover, nephropathy diabetic rats administrated with 15 μ l/g BW of beet root juice had significantly high CAT, GSH.px and SOD compering to nephropathy diabetic rats administrated with 10 μ l/g BW of beet root juice whereas, the level of MDA , All of these results due to bioactive polyphenol compounds in beet root juice, which play a vital role against lipid peroxidation. This is consistent with the results of **Georgiev *et al.*, (2010)** and **El- Gamal *et al.*, (2014)** showed that beet root juice diminishes the elevation in MDA level and restored the renal endogenous antioxidant CAT, GSH.px and SOD levels.

Furthermore, found that administration of rats with 8 ml/kg/day of red beet juice for 4 weeks led to protect them from oxidative stress and related damage by reducing the lipid peroxidation and increasing the antioxidant enzymes activities (catalase, glutathione peroxidase and SOD) (**Kujawska *et al.*, 2009**) .

BVEE beverage (250 and 500 mg/kg, p.o) was also suggested to be practical on kidney lipid peroxidation factors. The activity level of catalase, as an important antioxidant enzymes, was reduced by 27.97% following GM treatment, and notably increased by 83.92 and 92.62%, respectively, following the administration of 250 and 500 mg/kg BVEE. Similar trend was present for NP- SH content (non- protein sulphydryl- for the measurement of renal non-protein sulphydryl); a reduction of 37.94% following 85 mg/kg GM treatment was present, comparing to 71 and 81.71% increase in 250 and 500 mg/kg BVEE, respectively. The total protein content of GM treated animals was decreased by 71.46% in comparison with a dose dependent increase in pretreated groups of 250 mg/kg and 500 mg/kg BVEE by 37.35 and 43.74%, respectively (**El Gamal *et al.*, 2014**) .

Table 5: Effect of Fresh and Fermented Beet Root (*Beta vulgaris L.*) Juice on Lipid Peroxidation and Antioxidant Enzymes on rats with induced-colon cancer.

| Parameters Groups | | Parameter as Mean \pm SD | | | |
|---------------------------|-----|----------------------------|----------------------|------------------------|----------------------|
| | | MDA (n.mol/mg protein) | CAT (U/ mg protein) | GSH (mmol/ mg protein) | GPx (U/ gT protein) |
| Negative control group | | 0.780 \pm 0.010 | 3.665 \pm 0.085 | 4.375 \pm 0.085 | 5.730 \pm 0.260 |
| Positive control group | | 4.485 \pm 0.335*** | 0.755 \pm 0.015*** | 0.940 \pm 0.010*** | 1.085 \pm 0.025*** |
| Fresh beet root juice | 3ml | 3.635 \pm 0.125NS | 1.370 \pm 0.050NS | 1.800 \pm 0.250NS | 2.760 \pm 0.110NS |
| | 5ml | 1.935 \pm 0.175* | 1.895 \pm 0.135* | 2.940 \pm 0.180* | 4.010 \pm 0.140* |
| Fermented beet root juice | 3ml | 0.830 \pm 0.010** | 3.395 \pm 0.035** | 4.295 \pm 0.025** | 5.450 \pm 0.130** |
| | 5ml | 0.800 \pm 0.05*** | 3.635 \pm 0.305*** | 4.025 \pm 0.18*** | 5.520 \pm 0.060*** |

- Results are expressed as mean \pm SD
- Means with different superscript stars in the column are significantly different at significant * $p > 0.05$ * highly significant ($p < 0.01$); ** very highly significant ($p < 0.001$) and Ns. none significant as compared to group 2 positive group.
- MDA (Malondialdehyde) , CAT (Catalase) , GSH (Glutathione) and GPx (Glutathione peroxidase).

Table 6: Fermentation Data of Beet Root (*Beta vulgaris L.*) Juice

| Sample | Ph | Lactic (g/L) | Acetic (g/L) | Succinic (g/L) | Residual Glucose (g/L) | |
|---------|---------------|---------------|----------------|----------------|------------------------|---------------------|
| BRF-T0 | 6.5 \pm .02 | 0.2 \pm .09 | 0.05 \pm .13 | 0.02 \pm .08 | 30.0 \pm .16 | Before fermentation |
| BRF-T48 | 3.6 \pm .14 | 7.8 \pm .06 | 0.6 \pm .02 | 0.3 \pm .05 | 2.1 \pm .08 | After 48hr |
| BRF-T72 | 3.5 \pm .11 | 9.1 \pm .12 | 0.8 \pm .15 | 0.4 \pm .17 | 0.8 \pm .01 | After 72hr |

The data clearly shows a strong correlation between the consumption of residual glucose and the production of organic acids, primarily lactic acid. This metabolic activity led to a significant and rapid drop in pH, from a neutral 6.5 at T0 to an acidic 3.5-3.6 by T48. This acidic environment is inhibitory to many spoilage microorganisms and is a key goal of fermentation for preservation.

1- Detailed Parameter Analysis

A. pH and Acidity

- Observation: The pH dropped dramatically from 6.5 (T0) to 3.6 (T48) and stabilized at 3.5 (T72).
- Interpretation: This sharp decline is a direct result of the accumulation of organic acids, particularly lactic acid. A pH below 4.0 is generally considered a critical threshold for the preservation of fermented foods, as it prevents the growth of pathogenic and spoilage bacteria (**Adams and Moss, 2008**). The stabilization of pH between T48 and T72 suggests that the microbial activity may have slowed as glucose became scarce.

B. Residual Glucose

- Observation: Glucose was rapidly consumed, decreasing from 30.0 g/L at T0 to 2.1 g/L at T48 and further to 0.8 g/L at T72.
- Interpretation: Glucose serves as the primary energy source for the fermenting microorganisms. Its near-depletion by T72 indicates a highly active fermentation process. The low level of residual sugar at T72 also explains why the production of acids began to plateau, as the microbes were running out of their main substrate.

C. Lactic Acid Production

- Observation: Lactic acid concentration increased substantially from 0.2 g/L to 7.8 g/L at T48 and reached 9.1 g/L at T72. It is the major acid produced.
- Interpretation: The predominant production of lactic acid suggests that the fermentation was carried out mainly by homofermentative lactic acid bacteria (LAB). Homofermentative LAB, such as many *Lactobacillus* species, convert a large majority (over 85%) of glucose into lactic acid via the glycolytic pathway (Embden-Meyerhof-Parnas pathway) (**Madigan *et al.*, 2021**). This efficiency is desirable for rapid acidification.

D. Acetic and Succinic Acid Production

- Observation: Minor amounts of acetic acid (0.05 g/L to 0.8 g/L) and succinic acid (0.02 g/L to 0.4 g/L) were also produced.
- Interpretation: The presence of these acids indicates some metabolic diversity. Acetic acid can be produced through several pathways:

1. Heterolactic Fermentation: Some LAB (e.g., *Leuconostoc* spp.) are heterofermentative, producing lactic acid, acetic acid, and CO₂ from glucose.
2. Metabolism of Citrate or Other Compounds: If the substrate (BRF) contained other fermentable compounds, they could be converted to acetic acid.
3. Aerobic Conditions: Under limited oxygen, some LAB can produce acetate (Axelsson, 2004) .

Succinic acid is often a minor end-product in certain LAB fermentations and can contribute to the overall flavor complexity.

2. Biological and Practical Implications

The progression from T0 to T72 depicts a classic microbial succession. The rapid acidification created by homofermentative LAB not only preserves the product but also shapes its sensory properties. The tangy, sour taste is directly attributable to the high lactic acid content, while the small amounts of acetic and succinic acid likely contribute subtle notes to the overall flavor profile.

The fact that acid production slowed between T48 and T72, with only a modest increase in lactic acid (from 7.8 to 9.1 g/L) compared to the previous period, suggests that the fermentation was nearing completion by T72 due to glucose limitation. This is a critical point for determining the optimal fermentation endpoint to achieve the desired balance of acidity, flavor, and shelf-life.

4.6. Histopathological Report

The histopathological examination of colon sections of the control colonic wall showed intact mucosa with normal glands lined by mucous secreting cells, intact sub-mucosa and tunica muscosa (**Photos .A**). In contrast, we showed colon cancer in G2 group characterized by ulcerated mucosa, glands lined by slightly basophilic cells with elongated nuclei, extensive pleomorphic cells, sever interstitial inflammatory infiltrate in submucosa layer (**Photos. B**). Moreover, we showed in G3 group sever histopathological changes of colon mucosa characterized by complete sloughing of tunica mucosa , degeneration of intestinal gland, mononuclear inflammatory cells infiltrations and thick layer of mucous exudate (**Photos. C**). On the other hand, we showed mild improvement of histopathological alterations of colon layers in G4 group including desquamation and sloughing of tunica mucosa, mucous exudate mixed with inflammatory cells and degeneration of intestinal gland if compared with G1

group (**Photos. D**). While the G5 group revealed moderate improvement of histopathological changes of colon mucosa as superficial destruction of mucosa layer , presences of cell debris mixed with bluish exudate and moderate inflammation (**Photos. E**). Furthermore , the colon layer observed marked improvement of pathological lesions in G6 group including superficial regeneration of tunica mucosa and apparently health of intestinal gland as well as mild inflammation mixed with edema (**Photos. F**).

The histopathological findings in this study are similar to what was reported on ethanolic beetroot extract ameliorating gentamicin-induced nephrotoxicity in rats (**El Gamal *et al.*,2014**).

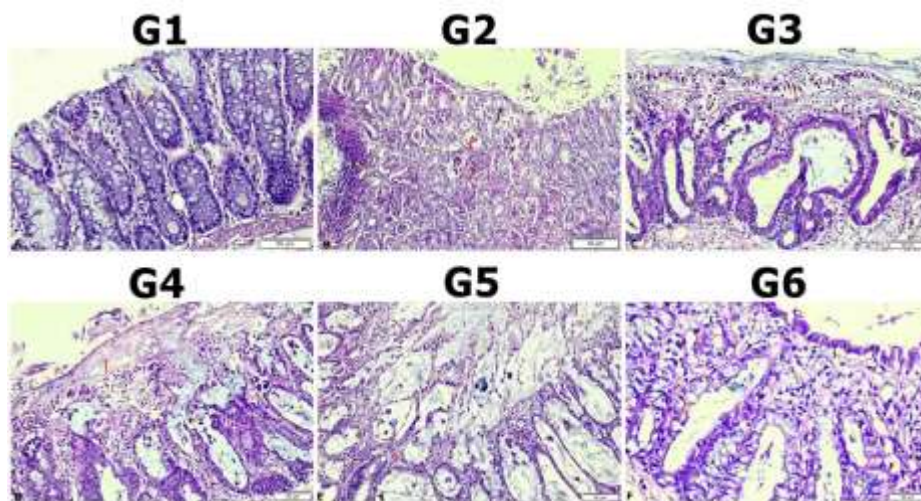


Fig1 . Histopathological examination of colon sections showing : (A) intact mucosa (black arrow) with normal glands lined by mucous secreting cells (yellow arrow). (B) ulcerated mucosa (black arrow), glands lined by slightly basophilic cells with elongated nuclei (red arrow), extensive pleomorphic cells, sever interstitial inflammatory infiltrate in submucosa layer (yellow arrow). (C) complete sloughing of tunica mucosa (red arrow) , degeneration of intestinal gland (black arrow), mononuclear inflammatory cells infiltrations and thick layer of mucous exudate (yellow arrow). (D) including desquamation and sloughing of tunica mucosa (black arrow), mucous exudate mixed with inflammatory cells (red arrow) and degeneration of intestinal gland (yellow arrow). (E) superficial destruction of mucosa layer (black arrow), presences of

cell debris mixed with bluish exudate (yellow arrow) and moderate inflammation (red arrow). (F) superficial regeneration of tunica mucosa (black arrow) and apparently health of intestinal gland (red arrow) as well as mild inflammation mixed with edema (blue arrow). (H&E, x200).

DMH is a toxic environmental pollutant, which was reported as a specific colon procarcinogen. The experimental colonic tumors induced by DMH in animals were of epithelial origin with a similar histology, morphology and anatomy to human colonic neoplasms. This pro-carcinogen could provide a sufficient model for studying colorectal cancer (Wang *et al.*, 2004).

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تقييم جذور البنجر الطازج والمخمّر كعامل علاجي في سرطان القولون والمستقيم التجريبي لدى الفئران

أمل فوزي الجزار ، نيفين سيوفي اسماعيل ، مروة طارق ابراهيم
سهيلة ناصر عبد المجيد

المستخلص العربي

يُعدّ عصير الشمندر الأحمر (*Beta vulgaris* L.)، المنتمي إلى الفصيلة الرمرامية (Chenopodiaceae)، غنيًا بالألياف الغذائية والمعادن (المنغنيز، المغنيسيوم، البوتاسيوم، الصوديوم، الفوسفور، الحديد، الزنك، النحاس، البورون، السيليكا والسيلينيوم)، إضافةً إلى فيتامينات مجموعة B المركبة. يُعرّف السرطان بأنه مرض يتميز بانقسام غير طبيعي ومستمر للخلايا. وعندما يحدث في القولون أو المستقيم، يُعرّف بسرطان القولون والمستقيم. هدفت الدراسة الحالية إلى استكشاف التأثير المحتمل المضاد للسرطان لعصير الشمندر الأحمر الطازج والمخمّر على الفئران المصابة بسرطان القولون. تم استخدام خمسين من ذكور الجرذان البيضاء الألبينو، وجرى تقسيمها إلى ست مجموعات، بواقع ثمانية جرذان لكل مجموعة: المجموعة الأولى (المجموعة الضابطة السلبية): تغذت على الغذاء الأساسي فقط. المجموعة الثانية (المجموعة الضابطة الإيجابية): حُقنت تحت الجلد مرة أسبوعيًا بجرعة ٣٥ ملغم/كغم من وزن الجسم من مادة DMH خلال فترة التجربة (١٢ أسبوعًا). المجموعتان الثالثة والرابعة: حُقنتا أسبوعيًا بجرعة ٣٥ ملغم/كغم من DMH وتلقيتا عن طريق الفم ٣ و ٥ مل من عصير الشمندر الأحمر الطازج خلال فترة التجربة. المجموعتان الخامسة والسادسة: حُقنتا أسبوعيًا بجرعة ٣٥ ملغم/كغم من DMH وتلقيتا عن طريق الفم ٣ و ٥ مل من عصير الشمندر الأحمر المخمّر خلال فترة التجربة. أظهرت نتائج المجموعات المعالجة بعصير الشمندر (الطازج والمخمّر) مع الغذاء الأساسي انخفاضًا معنويًا ($p < 0.05$) في أنشطة إنزيمات AST و ALT و ALP، ومستويات المصل من AFP و $\text{TNF-}\alpha$ و $\text{NF-}\kappa\text{B}$ و CEA و CA، إضافةً إلى اليوريا والكرياتينين وحمض اليوريك وMDA، مع ارتفاع مستويات المصل من البروتين الكلي والألبومين، وزيادة نشاط إنزيمات مضادات الأكسدة (GSH، CAT، GPx) مقارنةً مع المجموعة (٢) المصابة بسرطان القولون غير المعالجة (الضابطة الإيجابية). كما أظهر الفحص النسيجي المرضي تحسنًا واضحًا في مقاطع القولون لدى المجموعات (٣، ٤، ٥، ٦) التي تلقت ٣ و ٥ مل من عصير الشمندر الأحمر الطازج أو المخمّر. وأخيرًا، أوضحت الدراسة الحالية أن عصير الشمندر الأحمر يمكن أن يُحسن مؤشرات وظائف الكبد (ALT، AST، ALP) والمستويات المصلية لكل من AFP، $\text{TNF-}\alpha$ ، $\text{NF-}\kappa\text{B}$ ، CEA، CA، وكذلك مؤشرات وظائف الكلى (اليوريا، الكرياتينين، حمض اليوريك)، إضافةً إلى خفض مستويات MDA، وزيادة نشاط إنزيمات مضادات الأكسدة (GSH، CAT، GPx).

الكلمات المفتاحية: جذر البنجر ، ثنائي ميثيل هيدانتوين ، مضادات الأكسدة ، سرطان القولون .