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Potential Anticancer Effect of Cape Gooseberry Fruit Juice on Rats with Induced-Colon Cancer

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ABSTRACT

Colon cancer is one of the most common cancers influencing the gastrointestinal tract and the second leading cause of cancer deaths. Nowadays, non-traditional fruits such as Cape gooseberry play an important role in human nutrition and as an excellent source of bioactive phytochemicals. This study was directed to explore the potential anticancer effect of Cape gooseberry fruit on rats with colon cancer. Fifty male albino rats 6-8 weeks old and weighing 195 ±10 g were distributed into five groups of 10 rats each. Rats in group 1 were kept normal control and subcutaneously injected with 0.1 mL of the saline solution once weekly during the experimental period (12 weeks). Rats in groups 2, 3, 4, and 5 were subcutaneously injected once weekly with 35 mg/kg b. wt of 1, 2-Dimethylhydrazine (DMH) during the experimental period. Rats in group 2 were kept as Positive control. Rats in groups 3, 4, and 5 were given orally Cape gooseberry fruit juice (CGFJ) along with injected DMH. In comparison to normal control rats, administration of DMH caused significant decreases in body weight gain, and serum total protein and albumin levels as well as activities of GPx, CAT and GSH enzymes. Administration of CGFJ caused significant increases in serum levels of tumor biomarkers AFP, TNF-α, NF-κ β, UN, UA, Cr and MDA, as well as the total number of leukocytes, lymphocytes, monocytes and liver enzymes activities including AST, ALT and ALP. Oral administration of the different levels of CGFJ to rats injected with DMH caused significant amelioration in all of the above-mentioned parameters. The best results were obtained in the treated groups with high levels of CGFJ. The biochemical results were nearly consistent

with the histopathological findings, which confirmed the positive effect of the CGFJ against DMH-induced colon cancer. In conclusion, CGFJ exhibited antioxidant and antiinflammatory activities and protective effects against colon cancer and dysfunction of the liver and kidney in rats injected by DMH.

Keywords: Cape Gooseberry Fruit- Dimethylhydrazine- Colon Cancer- Anti-inflammatory- Antioxidant- Hepatorenal Functions- Histopathology

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**). CRC commonly initiates as a polyp (noncancerous tumor) and developed in the interior lining of the colon and/or rectum and multiplies slowly, over a period of 10 to 20 years (**Winawer and Zauber, 2002**). The most common type is a benign tumor formed by glandular structures in epithelial tissue (adenoma), which evolves into one or more adenomas in about one-third to one-half of persons (**Bond, 2000**). The likelihood that an adenoma will become cancerous increases as it becomes larger (**Pickhardt *et al.*, 2013**).

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**).

Cape gooseberry (*Physalis peruviana* L.,) is an edible fruit known as golden berry, a sort of cherry in the nightshade *Solanaceae* family. Cape gooseberry fruits (CGF) are annual perennials and have

acceptance flavor and appearance, with a sweet and sour taste. It is completely can adaptable to a wide variety of soils (**Ramadan and Mörsel, 2007**). It is a round orange-fleshed fruit, cherry-sized and loosely enclosed in a papery husk, which provides a natural wrapper for storing the fruit (**Wu *et al.*, 2004**).

Nowadays, CGF is one of the most important commercially grown fruits of the *Physalis* genus. Its foreign fruit has been winning recent popularity in the tropical and sub-tropical zone, Europe, Asia, Africa, and the Americas (**Singh *et al.*, 2019 and Thuy *et al.*, 2020**). The newly available of CGF might potentially as an indication of a food containing health-giving additives and medicinal benefit, an economically, rich source of bioactive phytochemicals and a potential method toward disease reduction (**Ramadan, 2011**). CGF is used in folk medicine for the treatment of several diseases such as malaria, asthma, hepatitis, dermatitis, diuretic, and rheumatism (**Wu *et al.*, 2004**). In addition, it is used as an anti-asthmatic, anti-septic, anti-ulcer, improvement of the optic nerve, treatment of throat inflammation and eliminating intestinal parasites, amoeba and reducing blood cholesterol level (**Arun and Asha, 2007**). The medicinal properties of Cape gooseberry have been attributed to their high antioxidant content (**Strik, 2007**).

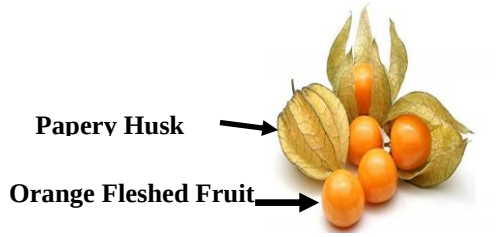
For this reason, more attention has been paid to Cape gooseberry fruit as a natural source of antioxidants as an endeavor to protect the human body from oxidative stress and hinder the progress of many chronic diseases, especially colon cancer. This study was directed to explore the potential anticancer effect of Cape gooseberry fruit on rats with colon cancer.

2. MATERIALS AND METHODS

2.1. Cape Gooseberry Fruit and Preparation of Juice:

Fully ripe Cape gooseberry fruit (*Physalis peruviana*, L) was collected from the farms near El-Beheira Governorate, Egypt. The general appearance of the whole fruit and berries of Cape gooseberry fruit is illustrated in **Figure 1**. Fresh fully ripe Cape gooseberry fruits were sorted, all papery husk removed, washed with cold running tap water and cut into small pieces. To obtain juice, pure fruits were blended for 3 min in a blender, then the strained to remove all seeds

and peels particles. The obtained Cape gooseberry fruit juice (CGFJ) was stored in a refrigerator till used.



Figur. 1: Whole Cape gooseberry fruits

2.2. 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.

2.3. Rats:

In the present study, fifty male albino rats 6-8 weeks old and weighing 195 ±10g 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.

2.4. Drugs and Chemicals:

A carcinogen 1, 2-Dimethylhydrazine (DMH) is a compound used experimentally to induce colon cancer in different animal models. It was 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.

2.5. Induction of Colon Cancer:

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

2.6. Grouping of Rats:

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

Group 1: Negative control group, rats were kept normal and subcutaneously 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 received orally 10% Cape gooseberry fruit juice (CGFJ) during the experimental period.

Group 4: Rats were injected once weekly with 35 mg/kg b. wt. of DMH and orally received 20% CGFJ during the experimental period.

Group 5: Rats were injected once weekly with 35 mg/kg b. wt. of DMH and orally received 30% CGFJ during the experimental period.

2.7. Feed Intake (FI) and Relative Body Weight Gain (RBWG %):

The daily feed intake (FI) of each rat was recorded and calculated based on the average daily intake during the experimental period. Body weight gain (BWG) was determined by the difference between the initial body weight of rats (IBW) and at the end of the experimental (FBW). Relative body weight gain (RBGW %) was estimated using the following equation:

Relative body weight gains (%) = Body weight gain/ IBW × 100

2.8. 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 were taken from the portal vein and divided into two

parts. The first part of the blood sample was 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 using a standard clinical hematology laboratory procedure as described by **Ruberto *et al.*, (2002)**.

The second taken part of the blood was collected in dry clean glass centrifuge tubes and allowed to coagulate at room temperature for serum separation. Serum was 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:

2.8.1. Screening of Serum Levels of AFP, TNF-α and NF-κ β:

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

2.8.2. Screening of Serum Activities of AST, ALT and ALP Enzymes:

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

2.8.3. Screening of Serum Total Protein and Albumin Levels:

Serum total protein (TP) and albumin (Alb) levels were determined calorimetrically using a spectrophotometer as described by **Burtis (1999)** and **Walker *et al.*, (1990)**, respectively.

2.8.4. Screening of Blood Urea, Uric Acid and Creatinine Levels:

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

2.8.5. Screening 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 according to the method described by **Mihara and Uchiyama (1978)**. Serum activity of glutathione peroxidase (GPx) enzyme was assayed by enzyme-linked immunosorbent assay as described by **Mannervik (1985)**. Activities of catalase (CAT) and glutathione (GSH) enzymes were assayed spectrophotometrically according to methods described by **Lartillot *et al.*, (1988)** and **Smith (1985)**, respectively.

2.9. Histopathological Screening:

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&E) stain for histopathological examination according to the technique described by **Bancroft and Gamble (2002)**.

2.10. Statistical Analysis:

The obtained data of all parameters were statistically analyzed using one-way analysis of variance (ANOVA) at the computerized SPSS software package (Version 16.0 for Windows). Data were expressed as means ± standard deviation (SD) and P-values <0.05 were considered significant.

3. RESULTS

The effect of oral administration of CGFJ on FI, FBW, BWG and RBWG% in DMH-induced colon cancer in rats are given in **Table 1**. It revealed that the treated rats with DMH alone have significant ($p<0.05$) reductions in FBW, BWG and RWG (%), while there is no significant change in FI, compared to normal control rats. Oral administration of Cape gooseberry fruit juice (CGFJ) combined with subcutaneous injection by DMH caused significant ($p<0.05$) increases in FBW, BWG and RWG (%) in comparison to injected rats by DMH alone. Preferable results in FBW, BWG and RWG (%) were found in groups given high levels of CGFJ.

Table 1: Effect of oral administration of CGFJ on FI, FBW, BWG and RBWG (%) in 1, 2-Dimethylhydrazine-induced colon cancer in rats.

Parameters / Groups	FI (g/day)	IBW (g)	FBW (g)	BWG (g)	RBWG (%)
Negative control group	14.93 ±0.25	202.43±0.12	415.10±0.10[a]	212.67±0.02[a]	105.06±0.97[a]
Positive control group	14.64 ±0.24	203.14±0.17	283.53±0.18[d]	80.39± 0.04[d]	39.57±0.74[d]
Treated rats with 10% CGFJ	14.71±0.24	203.00±0.29	389.53±0.19[c]	186.53±0.19[c]	91.87±0.78[c]
Treated rats with 20% CGFJ	14.79±0.27	202.00±0.29	404.53±1.13[b]	202.53±0.51[b]	100.26±0.43[b]
Treated rats with 30% CGFJ	14.57±0.23	203.14±0.17	407.10±1.17[b]	203.96±0.41[b]	100.04±0.28[b]

Each value represents the mean ± SD; Means with different letters in each column are significantly differs at $p< 0.05$; **CGFJ**: Cape gooseberry Fruit Juice; **FI**: feed Intake; **FBW**: Final Body Weight; **BWG**: Body Weight Gain; **RBWG**: Relative Body Weight Gain.

Results of serum concentration of AFP as a tumor biomarker, TNF-α as a pro-inflammatory cytokine and NF-κ β as a transcription factor are given in **Table 2**. The recorded results showed that subcutaneous administration of DMH alone caused significant ($p<0.05$) increases in serum concentration of AFP, TNF-α and NF-κ β as compared with untreated negative control rats. Oral

administration of the three different levels of CGFJ co-combined with the subcutaneous injection by DMH significantly reduced serum concentrations of AFP, TNF-α and NF-κ β, compared with the positive control group. According to the obtained results, priority is given to high levels of CGFJ for reducing the serum concentration of AFP, TNF-α and NF-κ β.

Table 2: Effect of oral administration of CGFJ on serum concentration of α-FP, TNF-α and NF-κ β in 1, 2-Dimethylhydrazine-induced colon cancer in rats.

Parameters / Groups	AFP (ng/ml	TNF- α (pg/ml)	TNF-κ β (ng/ml)
Negative control group	2.16 ± 0.05 ^{e}	1132±4.65^{e}	102.40 ± 2.11^{d}
Positive control group	8.92 ± 0.03^{a}	1367±1.25^{a}	121.00 ± 2.66^{a}
Treated rats with 10% CGFJ	5.22 ± 0.02^{b}	1280±1.65^{b}	111.30 ± 2.01^{b}
Treated rats with 20% CGFJ	4.95 ± 0.02^{c}	1186±2.94^{c}	105.30± 2.38^{c}
Treated rats with 30% CGFJ	3.66 ± 0.02 ^{d}	1170±2.68^{d}	104.50 ± 2.42^{c}

Each value represents the mean ± SD; Means with different letters in each column are significantly differs at p< 0.05; **CGFJ**: Cape Gooseberry Fruit Juice; **AFP**: Alpha-fetoprotein; **TNF-alpha**: tumor Necrosis Factor- Alpha; **TNF-*α***: Tumor Nuclear Factor-Kappa Beta (TNF-κ β).

The listed results in **Table 3** clarify that positive rats have a significant increase in the total number of leukocytes as well as total number and percent of lymphocytes and monocytes, compared to that of negative control rats. Treated rats by oral administration of CGFJ combined with subcutaneous injection by DMH have significant ($p<0.05$) reductions in the above mentioned parameters as compared to administrated rats by DMH alone. As mentioned above - the best results were reported in rats treated by high levels of CGFJ.

The results in **Table 4** illustrate the effect of oral administration of CGFJ on serum activities of AST, ALT and ALP enzymes in DMH-induced colon cancer in rats. Tabulated results revealed that DMH induced liver dysfunction as noted by the significant ($P<0.05$) increase in serum activities of AST, ALT and ALP enzymes, compared to the control negative group. Treatment of rats by oral administration of CGFJ co-incorporated with DMH caused a significant ($p<0.05$) reduction in serum activities of AST, ALT and ALP enzymes, compared to the positive group. The best results are obtained in groups treated by oral administration CGFJ at higher

levels along with DMH, compared to those that received a lower level of CGFJ.

As shown in **Table 5**, serum concentration of TP and Alb reduced in treated rats by DMH alone, compared to negative control rats. The combined oral administration of CGFJ with subcutaneous injection of DMH significantly ($P<0.05$) ameliorated serum levels of TP and Alb as compared with that treated by DMH alone.

Table 3: Effect of oral administration of CGFJ on a total number of leukocytes, lymphocytes and monocytes in 1, 2-Dimethylhydrazine-induced colon cancer in rats.

Parameters / Groups	Total number of leukocytes $\times 10^9$/L	Lymphocytes		Monocytes	
		$\times 10^9$/L	%	$\times 10^9$ /L	%
Negative control group	17.97±0.06^{d}	12.47±0.09^{d}	63.47±0.07^{d}	1.03±0.02^{e}	6.28±0.20^{d}
Positive control group	21.06±0.06^{a}	13.60±0.06^{a}	68.98±0.34^{a}	2.15±0.15^{a}	10. 8±0.21^{a}
Treated rats with 10% CGFJ	19.85±0.13^{b}	13.14±0.13^{b}	68.73±0.25^{b}	1.85±0.07^{b}	8.62±0.10^{b}
Treated rats with 20% CGFJ	18.95±0.05^{c}	12.94±0.05^{c}	68.57±0.13^{b}	1.81±0.01^{c}	7.56±0.08^{bc}
Treated rats with 30% CGFJ	17.98±0.10^{d}	12.55±0.18^{d}	64.58±0.18^{c}	1.28±0.05^{d}	6.62±0.12^{cd}

Each value represents the mean ± SD; Means with different letters in each column are significantly differs at $p< 0.05$; **CGFJ**: Cape Gooseberry Fruit Juice.

Table 4: Effect of oral administration of CGFJ on serum activities of AST, ALT and ALP enzymes in 1, 2-Dimethylhydrazine-induced colon cancer in rats.

Parameters / Groups	AST (µ/L)	ALT (µ/L)	ALP (u/L)
Negative control group	100.63±1.07^{e}	47.08±1.33^{e}	59.69±1.91^{e}
Positive control group	185.66±4.24^{a}	95.86±3.16^{a}	158.33±1.43^{a}
Treated rats with 10% CGFJ	141.10±1.57^{b}	77.63±1.05^{b}	120.23±3.32^{b}
Treated rats with 20% CGFJ	132.63±3.44^{c}	63.93±1.05^{c}	103.06±1.77^{c}
Treated rats with 30% CGFJ	121.86±1.56^{d}	56.06±1.81^{d}	86.06±2.08^{d}

Each value represents the mean ± SD; Means with different letters in each column are significantly differs at $p< 0.05$; **CGFJ**: Cape Gooseberry Fruit Juice; **AST:** Aspartate aminotransferase; **ALT**: Alanine aminotransferase; **ALP**: Alkaline phosphatase.

Table 5: Effect of oral administration of CGFJ on serum levels of TP and Alb in Dimethylhydrazine-induced colon cancer in rats.

Parameters / Groups	TP (g/dL)	Alb (g/dL)
Negative control group	8.18±0.11[a]	5.59±0.11[a]
Positive control group	3.54±0.22[e]	1.20±0.11[d]
Treated rats with 10% CGFJ	4.56±0.30[d]	2.53±0.01[c]
Treated rats with 20% CGFJ	5.82±0.24[c]	2.80±0.13[c]
Treated rats with 30% CGFJ	7.47±0.22[b]	3.86±0.13[b]

Each value represents the mean ± SD; Means with different letters in each column are significantly differs at $p< 0.05$; **CGFJ**: Cape gooseberry fruit Juice; **TP**: Total protein; **Alb**: Albumin

Results in **Table 6** demonstrate the effect of oral administration of CGFJ on serum levels of UN, UA and Cr in DMH-induced colon cancer in rats. The results showed a significant increase in serum BUN, DU and Cr concentrations in rats treated with DMH (positive control group) compared to normal rats. The results showed that there was a significant deficiency in serum UN, UA and Cr levels of the DMH-treated groups with different levels of CGFJ in combination, compared to the treated rats with DMH alone. The results showed that the oral administration of CGFJ at the highest level improved serum levels of BUN, UA and Cr in DMH-treated rats.

Table 6: Effect of oral administration of CGFJ on serum levels of UN, UA and Cr in 1,2 Dimethylhydrazine-induced colon cancer in rats

Parameters / Groups	UN (mg/dL)	UA (mg/dL)	Cr (mg/dL)
Negative control group	52.07±1.22[d]	1.22±0.04[d]	0.418±0.03[d]
Positive control group	81.17±1.52[a]	2.37±0.01[a]	0.880±0.03[a]
Treated rats with 10% CGFJ	72.53±0.60[b]	2.04±0.20[b]	0.767±0.02[b]
Treated rats with 20% CGFJ	68.50±2.80[b]	1.61±0.05[c]	0.710±0.008[b]
Treated rats with 30% CGFJ	59.03±2.56[c]	1.46±0.02c[d]	0.557±0.02[c]

Each value represents the mean ± SD; Means with different letters in each column are significantly differs at $p< 0.05$; **CGFJ**: Cape gooseberry Fruit Juice; **UN:** Urea nitrogen; **UA:** Uric acid; **Cr:** Creatinine.

Tables 7 represent the serum level of MDA and activities of antioxidant enzymes (GPx, CAT and GSH) in normal rats, subcutaneously injected rats with DMH alone and treated along with oral administration of CGFJ. In comparison to the negative control

rats, administration of DMH caused a significant increase in serum MDA level and decreased activities of antioxidant enzymes. Oral administration of the different levels of CGFJ to injected rats with DMH caused significant amelioration in serum MDA level and activities of GPx, CAT and GSH enzymes when compared to the positive control group. The best results in serum MDA levels and activities of antioxidant enzymes were found in the treated groups by the upper levels of CGFJ.

Table 7: Effect of oral administration of CGFJ serum levels of MDA and activities of antioxidant enzymes (GPx, CAT and GSH) in 1,2-Dimethylhydrazine-induced colon cancer in rats.

Parameters / Groups	MDA (µmol/ml)	GPX (U/mg)	CAT (ng/mg)	GSH (U/mg)
Negative control group	11.05±0.25^{d}	57.84±2.67^{a}	131.27±2.63^{a}	37.27±0.81^{a}
Positive control group	20.37±1.42^{a}	22.41±0.22^{e}	62.20±2.46^{d}	23.42±1.13^{d}
Treated rats with 10% CGFJ	17.50±0.61^{b}	36.16±1.65^{d}	89.20±2.90^{c}	28.00±0.91^{c}
Treated rats with 20% CGFJ	15.67±0.37b^{c}	42.39±1.24^{c}	114.82±2.19^{b}	31.95±0.89^{b}
Treated rats with 30% CGFJ	13.72±0.27^{c}	50.42±1.00^{b}	121.62±1.72^{b}	33.57±1.64^{b}

Each value represents the mean ± SD; Means with different letters in each column are significantly differs at $p< 0.05$; **CGFJ**: Cape Gooseberry fruit Juice; **MDA**: Malondialdehyde; **GPx**: Glutathione peroxidase; **CAT**: Catalase; **GSH**: Glutathione

Results of Histopathological Examination:

Microscopic examination of colon tissue samples from the negative control group as shown in **Figs. 2 and 3** revealed a normal histological structure of colon mucosa that contained numerous mucous glands and showed a simple columnar epithelium lining. Colon sections from the positive control group showed marked histopathological alterations as intense inflammatory reactions and wide areas of ulceration **(Figs. 4 and 5**) as well as the glands showed cystic dilation and marked dysplastic changes (**Fig 6**). Mild improvement was detected by administration of DMH + 20% of CGFJ as only areas of ulcerations were frequently observed in the

examined sections with marked inflammatory edema in the submucosa (**(Figs. 7)** as well as mild glandular dilatation and glandular dysplasia (**Fig. 8**). Additionally, moderate improvement was shown in colon sections of rats treated with DMH+ 20% of CGFJ showed moderate submucosal edema and dysplasia in glands as illustrated in **Fig (9)** and **(10)**, respectively. Photomicrograph of treated rats with DMH + 30% of CGFJ revealed only mild mononuclear inflammatory cell infiltration in some sections (**Fig 11**) and apparently normal mucosa (**Fig 12)**.

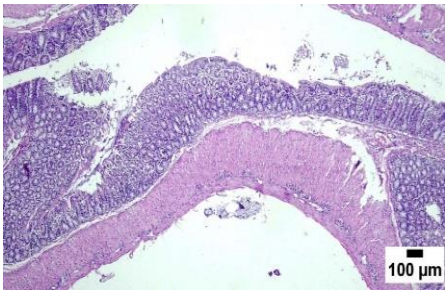


Fig. (2): Photomicrograph of colon from normal rats showing normal colon wall (H&E).

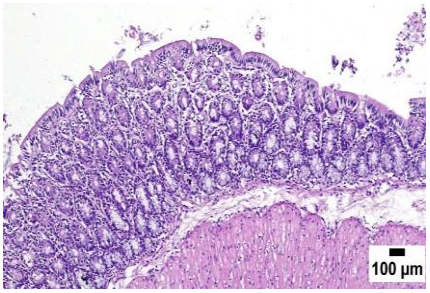


Fig. (3): Photomicrograph of colon from normal rats showing normal colon mucosa that contained numerous mucous glands (H&E).

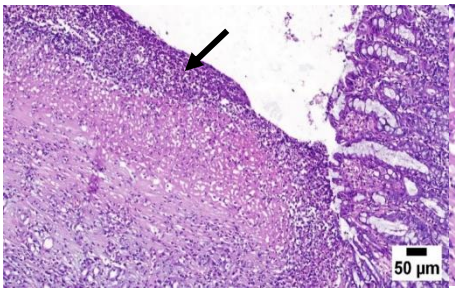


Fig. (4): Photomicrograph of colon from treated rats with DMH alone showing complete sloughing of mucosa with intense inflammatory cells infiltration (arrow) (H&E).

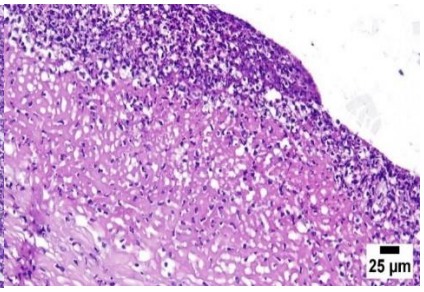


Fig. (5): Photomicrograph of colon from treated rats with DMH alone showing ulceration with complete sloughing of mucosa with intense inflammatory cells infiltration (H&E).

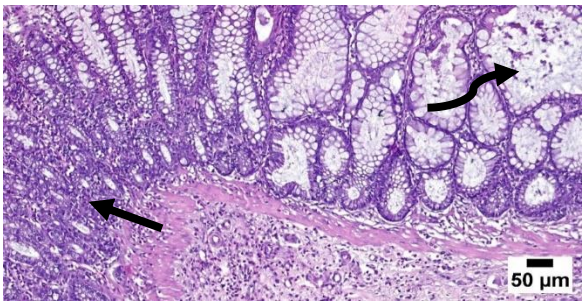


Fig. (6): Photomicrograph of colon from treated rats with DMH alone showing dysplasia in glandular epithelium (straight arrow) with cystic dilatation (curved arrow) (H&E).

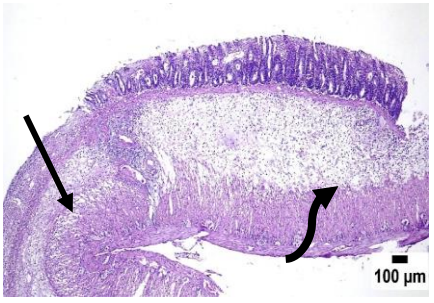


Fig. (7): Photomicrograph of colon from treated rats with DMH + 10% of CGFJ showing areas of ulceration (straight arrow) and marked inflammatory edema in the submucosa (curved arrow) (H&E).

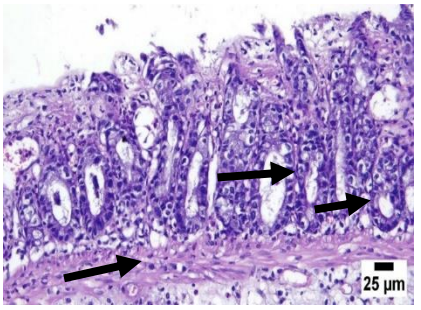


Fig. (8): Photomicrograph of colon from treated rats with DMH + 10% of CGFJ showing mild glandular dysplasia (arrows) (H&E).

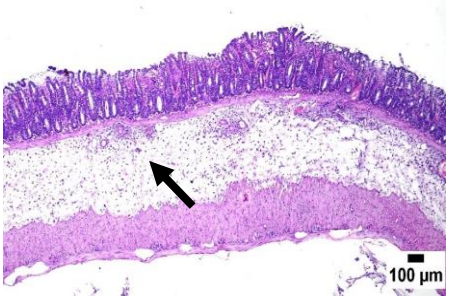


Fig. (9): Photomicrograph of colon from treated rats with DMH + 20% of CGFJ showing moderate submucosal edema (arrow) (H&E).

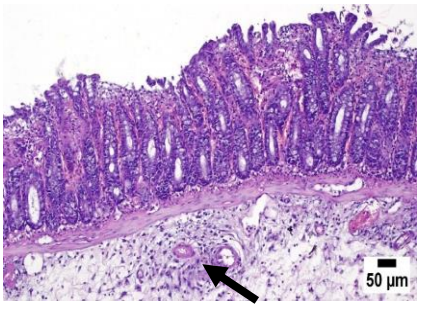


Fig. (10): Photomicrograph of colon from treated rats with DMH + 20% of CGFJ showing submucosal inflammatory edema (arrow) and dysplasia in glands (H&E).

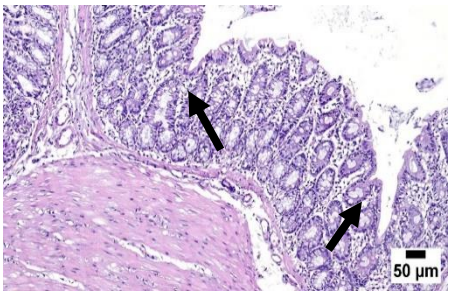


Fig. (11): Photomicrograph of colon from treated rats with DMH + 30% of CGFJ showing mild mononuclear inflammatory cells infiltration (arrows) (H&E).

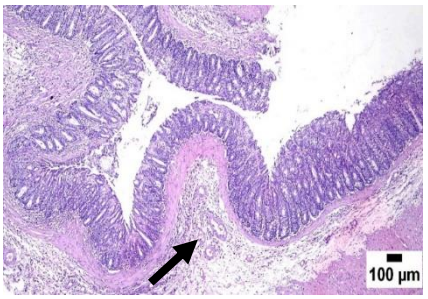


Fig. (12): Photomicrograph of colon from treated rats with DMH + 30% of CGFJ showing apparently normal glands with submucosal edema (arrow) (H&E).

4. DISCUSSION:

DMH is a procarcinogen used in the formation of colon and rectum selectively tumors in rats by IP injections (**Reddy, 2002**). This study was directed to explore the potential anticancer effect of Cape gooseberry fruit on rats with colon cancer. Belonging to our results, we discovered that the intoxication of rats by DMH generated neoplastic lesions of the colon with a significant decrease in BWG (g), RWG (%), TP, Alb, and GP-x, CAT, and GSH. As well the elevation in serum concentrations of AFP, TNF-α, TNE- NF-κ β, UN, UA, Cr, MDA, and the total numbers of leukocytes, lymphocytes and monocytes and activities of AST, ALT, and ALP enzymes. These results are proportionate with the results gained from **Rajeshkumar and Kuttan, (2003)** who showed that the administration of DMH induced elevation in the liver, kidney, and serum lipid peroxidation and serum total bilirubin levels. Also, **Sengottuvelan *et al.*, (2006)** reported that DMH treatment decreases the level of antioxidant enzymes. As well, **Ahmad and Sultan, (2012)** informed that the injection with DMH caused oxidative stress and an initial inflammatory and tumor encouragement response in the Wistar rats' colon. The serum level of MDA and proinflammatory cytokines was remarkably increased, while GSH, GPx, GR, and GST were decreased significantly in the DMH-treated group.

Recently, **Thangaraja *et al.*, (2018)** and **Punvittayagul *et al.*, (2021)** mentioned that there was a significant decrease in the FBW

and a rise in serum levels of biochemical markers of liver functions in the rats with DMH, suggesting that DMH-induced hepatic cell deterioration. **Rajeshkumar and Kuttan, (2003)** illustrated that DMH is metabolically turned on in the liver by an intermediary reaction chain through azoxymethane and methylazoxymethanol to the ultimate cancerogenic metabolite. Where highly reactive methyldiazonium ion alkyls DNA encourages gene alteration and break of DNA chain in multiple organs especially the animal's liver. Therefore, the action mechanism DMH might be related largely to DNA methylation of the colon cell stem placed at the crypt base in the intestines, with the development of colon adenocarcinoma (**Umesalma and Sudhandiran, 2010**). Additionally, **De-Souza and Costa-Casagrande, (2018)** mentioned that DMH provokes colon tumor growth via cellular oxidative damage and up-regulation of tumorigenic pathways such as Wnt andPI3K/Akt. As reported by **Shojaei-Zarghani *et al.*, (2020**). Akt is a crucial player in colon carcinoigenesis, inhibits the GSK3β/APC/auxin-mediated degradation of β-catenine and develops β-catenin target oncogenes (c-Myc and cyclin D1).

Our results exhibit that CGFJ had a protective mechanism against the dangers of oxidative stress as well as anticancer effects caused by DMH in rats. These results were corroborated by biochemical analysis and histopathological examination. This result was nearly similar to that of **Ramadan *et al.*, (2015)** who concluded that Cape gooseberry increased BWG and food efficiency ratio, impeded lipid peroxidation, and improved hepatorenal functions and blood picture, as well as was more potent in inhibiting colon cell lines (IC 50: 142 µg/ml) in rats.

The biochemical results were nearly consistent with the histopathological findings. Additionally, these findings agree to some extent with those of **Hassan *et al.*, (2017)** who declared that the giving of CGFJ to rats with hepatocarcinoma induced by Dimethylnitrosamine-(DENA) and CCl4 was related to the diminishing the elevation in serum concentrations of AFP, MDA, AST, ALT and ALP, as well as the improvement in the antioxidant activity of GSH, TAC, SOD and CAT, compare to positive rats. Moreover, **Badr and Naeem (2019)** revealed that the addition of Cape gooseberry to the rats' diet with precarcinogenic aflatoxins

increased body weight and FI, and inhibited levels of lipid peroxidation and a tumor indicator. In addition to the improvement in lipid profiles, liver and kidney functions, serum levels of globulin, TP, Alb.

The beneficial health effects of Cape gooseberry fruit might be attributable to its content of multiple bioactive ingredients, which act or conflict with free radicals. Sequentially, Cape gooseberry fruit will hinder the deleterious toxic effects of DMH by scavenging free radicals and other ROS or by modulators of the inflammatory reaction. **Hassan and Ghoneim (2013)** informed that natural antioxidant compounds such as polyphenols and flavonoids are the system by which to improve antioxidant protection as an enabling index for antilipid peroxidative properties and antioxidant effects. Earlier studies indicated that CGJ is a substantial source of bioactive and antioxidant compounds (**Mokhtar *et al.*, 2018**) and a good source of an anticancer agent's activity (**Ramadan, 2011**). It is a good store for a variety of phenolic, flavonoid, and volatile compounds. Flavonoids are famous for their immune-modulator, anti-inflammatory effects, and inhibiting proinflammatory cytokine production and their receptors (**Kempuraj *et al.*, 2005**). Additionally, the anticancer, antioxidant and anti-inflammatory activities of Cape gooseberry fruit may be due to their riches in bioactive compounds such as bisabolol (**Baraga *et al.*, 2009**), monoterpene (**Narv*α*ez-Cuenca *et al.*, 2014**), carotenoids, vitamin C, kaempferol, quercetin, myricetin, routing, epicatechin and catechin (**Saavedra *et al.*, 2019**), and withanolides and phylloquinone (**Ramadan, 2019**). The natural compound 4-hydroxywithanolide E presented in Cape gooseberry fruit prevents the development of colon cancer monolayer and spheroid culture (**Park *et al.*, 2016**). As well, the Cape gooseberry fruit possesses a higher concentration of carotenoids (**Etzbach *et al.*, 2018**). Carotenoids have various important biological effects such as provitamin A, immunomodulatory, antioxidant, antidegenerative and anticancer (**Olivares-Tenorio *et al.*, 2016**).

5. CONCLUSION

Cape gooseberry fruit juice has anticancer, anti-inflammatory, hepatoprotective, antioxidant, and inhibited lipid peroxidation when

given orally to rats with experimentally-induced colon cancer. These effects could be related to the presence of bioactive constituents in the fruit. This study suggests that the intake of fresh Cape gooseberry fruit may be beneficial for patients who suffer from colon cancer. Despite this, we need more research on the efficacy of the fruit on humans

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