Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **44** Issue **S-5 Year 2023** Page **1823: 1830***

Anti-inflammatory and Antioxidant Effects of Grapefruit Juice on Ulcerative Colitis induced by Acetic Acid in Rats

Asmaa Mahmoud A. Mohamed[1], Mostafa A. Shalaby[2]*, Neveen S. Ismail[3]

[1]*Home Economic Department, Faculty of Specific Education, Aswan University, Egypt.*
[2]*Pharmacology Department, Faculty of Veterinary Medicine, Cairo University, Egypt.*
[3]*Nutrition and Food Science Department, Faculty of Home Economics, Helwan University, Egypt.*

Corresponding Author: Mostafa A. Shalaby

Article History

Received: 21 June 2023
Revised: 05 September 2023
Accepted: 03 November 2023

Abstract

Grapefruit is commonly consumed around the world due to its nutritional and medicinal properties. Ulcerative colitis (UC) accounts for an inflammatory bowel disease (IBD) accompanied by irritation, recurrent inflammation, and ulceration of the colon's mucosa. Our objective was to assess the antiinflammatory and antioxidant impacts of grapefruit juice (GFJ) in rat with UC induced by acetic acid (AA). There were five groups made up of 35 mature male rats. Group 2 served as a positive control with UC, whereas group 1 was preserved as a negative control. For 8 weeks, groups 3, 4, and 5 received UC while also given 2.5, 5 or 10% of GFJ orally. Feed efficiency ratio (FER), body weight growth (BWG), and feed intake (FI) were computed. From rat eye orbital plexuses blood samples were taken to separate the serum after centrifugation. The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity were assessed utilizing serum samples. The antioxidant enzymes catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) in serum were quantified. Serum inflammatory cytokines as tumor necrosis factor alpha (TNFα), interleukin 1 β (IL1 β), IL6 and IL8, as well as triglycerides (TG) and total cholesterol (TC) were measured. In liver homogenate, glutathione (GSH) and malondialdehyde (MDA) were determined. Additionally, histopathology of the colon was accomplished. Our findings specified that GFJ at 10% significantly decreased FI, BWG and FER, cytokines TNFα, IL1 β, IL6 and IL8 and TC and TG. Relative to the positive controls, the rats treated with GFJ exhibited elevated serum concentrations of SOD, GPx, and CAT enzymes. Liver GSH was elevated and MDA was reduced. Histopathological examination of the colon showed that GFJ at 10% reduced ulcerative colitis. Because grapefruit juice has strong anti-inflammatory and antioxidant characteristics and reduces oxidative stress and inflammation in rats, grapefruit juice, at a concentration of 10%, likely protects against ulcerative colitis. Patients with ulcerative colitis may find it helpful to consume grapefruit juice as a beverage.

Key words: *Grapefruit, Ulcerative colitis; Biochemical analysis, Anti-oxidant, Cytokines; Colon Histopathology.*

1. Introduction

One of the most popular fruits consumed worldwide are the citrus fruits. They are well-liked for both their flavor and nutritional contents. According to Castro-Vazquez et al. (2016), citrus fruits provide a rich supply of Vit C, fiber, and flavonoids like naringin, naringinin, and quercetin. One of the fruits that people eat the most frequently is grapefruit. Due to its antioxidant, antiinflammatory, and neuroprotective properties, naringin from grapefruit guards against epilepticus state in rats (Golechha et al. 2011)**.**

The health advantages of grapefruit juice, such as protection against inflammatory illnesses, cardiovascular diseases, diabetes, cancer, and neurodegenerative disorders, have increased interest in its bioactive constituents (Hwang et al. 2012 and Mendes et al. 2019). Fukuda et al. (1997), reported that the active ingredients in grapefruit juice include naringin, naringinin, limonin, and obacunone .

The inflammatory bowel diseases (IBD), a gastrointestinal tract persistent inflammatory disease, are marked by persistent recurrent colon ulceration (Khor et al. 2011). IBD is a collection of idiopathic chronic inflammation and ulceration affecting the large intestine. Ulcerative colitis (UC) and Crohn's disease (CD) are the main IBD kinds that are characterized by long-lasting inflammation of the GIT tract (Xia et al. 1998 and Balmus et al. 2016). UC, an inflammatory condition of the GIT (IBD), is widely acknowledged to involve a complex interplay between environmental and genetic parameters, particularly, bacteria that can disrupt the mucosal barriers, leading to the interaction of the luminal bacteria with the mucosal immune system (Scaldaferri and Fiocchi, 2007)**.**

UC is an idiopathic, recurrent, and autoimmune disease with recurrent episodes of damage, inflammation, irritation, and ulceration in the lining epithelium of the colon and its effects on the mucosa of the colon are of continued nature (Ng et al. 2013). The other type of IBD is CD, which is distinct patchy lesions likely dispersed within the GIT, without a specific pattern (Magro, 2017).

2. Material and Methods

Materials:

Grapefruits:

The Rutaceae family includes the genus Citrus, to which grapefruit belongs. Citrons, oranges, pomelos, limes, lemons, and mandarins (tangerines) are among the other members of the Citrus genus. Citrus fruits possess a unique characteristic of being classified as berries, featuring segmented internal structures. The photo of grapefruit is as follows:

Grapefruits

Grapefruit was obtained from a nearby market, then peeled and juiced with an electrical blender. The grapefruit juice (GFJ) was orally administered to rats via a stomach tube in 1 ml/rat at a concentration 2.5, 5, or 10% for 8 weeks.

Acetic acid (AA):

AA represents a liquid material with the potential to trigger UC. It is a monocarboxylic acid with simple structure of two carbon atoms. It has a chemical formula of CH3 COOH. A 5% solution (V/V) of AA was acquired from El-Gomhorya Pharmaceutical Company, Cairo, Egypt in 100ml bottles.

Basal diet:

The basal diet formulation followed the procedure of Reeves et al. (1993). It comprised 20 % casein (as a protein), 10 % carbohydrate, 5% fiber, 4.7% corn oil (as a fat), 3.5 % salt mixture, 2% choline chloride, and 1% vitamin mixture. The remaining portion, up to 100%, was composed of corn starch.

Rats:

35 rats of the Sprague-Dawley strain (220±5 g b.wt) and 8 months age were acquired from the Agriculture Research Center, Ministry of Agriculture, Egypt.

Ethical approval:

This study was approved according to rules of Faculty of Specific Education Committee, Aswan University, under Ethical Code of ASWU/S E/ Nutrition/ 23 / 05 dated 15/9/2023.

Methods:

Ulcerative colitis induction:

Rats were subjected to AA administration every 24 hours for a weak using a plastic catheter. 1 ml of AA (3%) was introduced to the rat's anus via a plastic dropper, and 15-minute period of positioning the rat in the Trendelenburg position was employed to trigger UC, as mentioned by Cagin et al. (2016) and Wang et al. (2019).

Experimental and grouping of rats:

35 Sprague Dawley strain rats have been accommodated within adequately aired cages, maintaining a room temperature (25±3 °C). The humidity level was set at 50% ± 5%, and the rats underwent a 12-hour light/dark cycle. The rats underwent 1-week adaption on AIN-93 basal diet, where water was provided ad libitum. The rats were allocated into 5 groups: group 1 fed a basal diet and served a negative control. Group 2 was the positive control and had UC induced by AA. Groups 3, 4, and, 5 were subjected to AA-triggered UC, followed by oral administration of GFJ (1ml /rat) at 2.5, 5, and 10%, respectively. The rats' weights were recorded at the start and conclusion of the dietary course. On a daily basis throughout the 8-week experimental episode, the rats' feed intake (FI) was documented. The calculation of body weight gain (BWG) was undertaken. The assessment of feed efficiency ratio (FER) and body weight gain percentage followed the methodology of Chapman et al. (1959) with the subsequent formulas:

Feed efficiency ratio (FER) = weight gain (g) ÷ feed consumed (g)

BWG % = final weight (g) – initial weight (g) ÷ initial weight (g) ×100

Upon the conclusion of the 8-week experimental period, the rats underwent an overnight fasting period before anesthetizing with sodium pentobarbital (Nesdonal®) at 50 mg/kg/rat. Clear serum samples were acquired by obtaining blood from the orbital plexuses of the eye from each rat and applying 15-minute centrifugation at 10.000 rpm. Livers were dissected out to prepare liver homogenates. The colons (large intestines) were excised and subsequently immersed within a solution of formaldehyde in a neutral buffer (10%) until used in histopathological inspection.

Biochemical analysis:

To estimate alanine aminotransferase (ALT) and aspartate aminotransferase (AST) serum levels, the technique of Bergmeyer et al. (1978) was adopted. The spectrophotometric determination of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) enzymes activities followed the protocols of Nishikimi et al. (1972), Racz et al. (1967), and Aebi (1984). Inflammatory cytokines TNFα, IL1β, IL6 and IL8 were appraised with enzyme linked immunosorbent assay (ELISA) as outlined by Sutton et al. (2006). The calorimetric assessment of serum triglycerides (TG) and total cholesterol (TC) followed the protocols of Wahlefeld (1974) and Allain et al. (1974).

Preparation of liver homogenate:

A liver sample weighing one gram was acquired from each rat and underwent washing within ice-cooled 0.9% NaCl, followed by homogenizing within ice-cooled 10 ml potassium chloride solution (1.15%) in buffer solution of 50 mM potassium phosphate (pH 7.4) to acquire hepatic homogenates of 10% (W/V). Liver homogenates underwent 15-minute of centrifugation at 9000 rpm before utilizing in assessment of hepatic reduced glutathione (GSH) and malondialdehyde (MDA), as outlined by Jollow et al. (1974) and Ohkawa et al. (1979).

Histopathological inspection:

The fixed specimens of large intestines underwent trimming, washing, and subsequent dehydration in rising concentrations of alcohol (70% to 100%). The colon was extracted and washed with saline. Small amounts of tissue of the colon were sectioned for staining. These specimens underwent heamatoxylin and eosin (H&E) staining before microscopic examination following Bancroft and Stevens (1977).

Statistical analysis:

Data were presented as means ± SD. SPSS program was utilized for statistical analysis employing one-way ANOVA test. Significance was adjusted at $P < 0.05$ according to Snedecor and Cochran (1986).

3. Results and Discussion

Table 1 shows that grapefruit juice at 2.5, 5.0 and 10 % in acetic acid-induced UC rats significantly decreased, in a dose dependent manner, FI, BWG%, and FER**.**

Table (1): Effect of grapefruit juice (GFJ) at 2.5, 5.0, and 10 % on FI, BWG percent, and FER in rats with UC (n=7 rats).

Parameters Groups	FI (g/day)	BWG (%)	FER
Negative control	23.0	12.69 ± 0.36^{a}	0.55 ± 0.001^{a}
Positive control	20.0	9.74 ± 0.21^{bc}	0.487 ± 0.002^{c}
GFJ at 2.5%	19.0	8.66 ± 0.20^{b}	0.455 ± 0.001^{b}
GFJ at 5%	16.5	7.69 ± 0.25^{c}	0.466 ± 0.002^{c}
GFJ at 10 %	13.3	6.89 ± 0.12^{d}	0.518 ± 0.006^{d}

In each column, the means ± S.D with distinct superscripted letters specify statistical significance at a $P < 0.05$.

Data in Table 2 showed that grapefruit juice at 2.5, 5.0, and 10% in acetic acid-induced UC rats significantly decreased, in a dosage reliant manner, the levels of serum AST and ALT.

Table (2): Effect of grapefruit juice (GFJ) at 2.5, 5.0, and 10 % on serum AST and ALT.

Parameters Groups	GPx (mg/dL)	SOD (mg/dL)	CAT (mg/dL)
Negative control	$85.12 \pm 0.22^{\mathbf{d}}$	$86.56 \pm 0.06^{\mathbf{d}}$	$24.05 \pm 0.46^{\mathbf{d}}$
Positive control	$75.05 \pm 0.34^{\mathbf{e}}$	$65.42 \pm 1.13^{\mathbf{e}}$	$16.03 \pm 1.12^{\mathbf{e}}$
GFJ at 2.5%	$92.71 \pm 0.28^{\mathbf{c}}$	$88.14 \pm 1.26^{\mathbf{c}}$	$20.94 \pm 0.63^{\mathbf{c}}$
GFJ at 5%	$98.14 \pm 0.80^{\mathbf{b}}$	$90.94 \pm 0.63^{\mathbf{b}}$	$21.55 \pm 0.63^{\mathbf{b}}$
GFJ at 10 %	$99.02 \pm 0.32^{\mathbf{a}}$	$95.14 \pm 0.40^{\mathbf{a}}$	$23.77 \pm 0.43^{\mathbf{a}}$

In each column, the means ± S.D with distinct superscripted letters specify statistical significance at a $P < 0.05$ level.

Compared to the positive controls, grapefruit juice at 2.5, 5.0 and 10 % in acetic acid-induced UC in rats caused significantly elevated serum antioxidant enzymes; SOD, GPx, and CAT (Table 3).

Table (3): Impact of grapefruit juice (GFJ) at 2.5, 5.0 and 10 % on antioxidant enzymes; GPx, SOD, and CAT in UC rats (n=7 rats).

Parameters Groups	GPx (mg/dL)	SOD (mg/dL)	CAT (mg/dL)
Negative control	$85.12 \pm 0.22^{\mathbf{d}}$	$86.56 \pm 0.06^{\mathbf{d}}$	$24.05 \pm 0.46^{\mathbf{d}}$
Positive control	$75.05 \pm 0.34^{\mathbf{e}}$	$65.42 \pm 1.13^{\mathbf{e}}$	$16.03 \pm 1.12^{\mathbf{e}}$
GFJ at 2.5%	$92.71 \pm 0.28^{\mathbf{c}}$	$88.14 \pm 1.26^{\mathbf{c}}$	$20.94 \pm 0.63^{\mathbf{c}}$
GFJ at 5%	$98.14 \pm 0.80^{\mathbf{b}}$	$90.94 \pm 0.63^{\mathbf{b}}$	$21.55 \pm 0.63^{\mathbf{b}}$
GFJ at 10 %	$99.02 \pm 0.32^{\mathbf{a}}$	$95.14 \pm 0.40^{\mathbf{a}}$	$23.77 \pm 0.43^{\mathbf{a}}$

In each column, the means ± S.D with distinct superscripted letters specify statistical significance at a $P < 0.05$ level.

According to Table 4, grapefruit juice (GFJ) at 2.5, 5.0, and 10% in acetic acid-induced UC rats significantly decreased, in a dosage reliant-manner, cytokines TNF α, IL-1β, IL6, and IL-8 relative to the positive controls.

Table (4): Table 4: Impact of grapefruit juice (GFJ) at 2.5, 5.0, and 10% on serum TNF α, IL-1β, IL6, and IL-8 in acetic acid-induce d UC in rats (n=7 rats).

Parameters Groups	GSH (nmol/mg protein)	MDA (nmol/mg protein)
Negative control	45.12 ± 0.22^{a}	57.57 ± 0.36^{e}
Positive control	29.85 ± 0.34^{e}	76.14 ± 1.88^{a}
GFJ at 2.5%	34.71 ± 0.28^{d}	70.72 ± 1.98^{b}
GFJ at 5%	39.14 ± 0.80^{c}	68.85 ± 1.56^{c}
GFJ at 10 %	42.28 ± 0.42^{b}	60.71 ± 0.92^{d}

In each column, the means ± S.D with distinct superscripted letters specify statistical significance at a P<0.05.

Our findings indicated that grapefruit juice (GFJ) at 2.5, 5.0, and 10% in acetic acid-induced UC in rats caused elevated GSH and lowered MDA relative to the positive controls (Table 5).

Table (5): Table 5: Impact of GFJ at 2.5, 5.0, and 10% on GSH and MDA in UC rats. (n=7 rats).

Parameters Groups	TNF α (Pg/ml)	IL1β (Pg/ml)	IL6 (Pg/ml)	IL8 (Pg/ml)
Negative control	118±2.30^{d}	56.28±1.20^{d}	54.08±2.20^{d}	75.57±2.63^{d}
Positive control	132±3.40^{a}	89.57±2.39^{a}	69.16±2.39^{a}	85.14±6.52^{a}
GFJ at 2.5%	127±2.50^{b}	66.42±3.80^{b}	56.42±1.80^{b}	79.71±4.91^{b}
GFJ at 5%	122±2.50^{c}	46.00±4.76^{c}	50.00±2.76^{c}	65.80±2.74^{c}
GFJ at 10 %	119±2.50^{c}	35.42±2.99^{c}	45.42±1.19^{c}	62.14±3.18^{c}

In each column, the means ± S.D with distinct superscripted letters specify statistical significance at a P < 0.05.

Grapefruit juice (GFJ) at 2.5, 5.0, and 10% significantly decreased TC and TG as compared to the positive controls (Fig. 1).

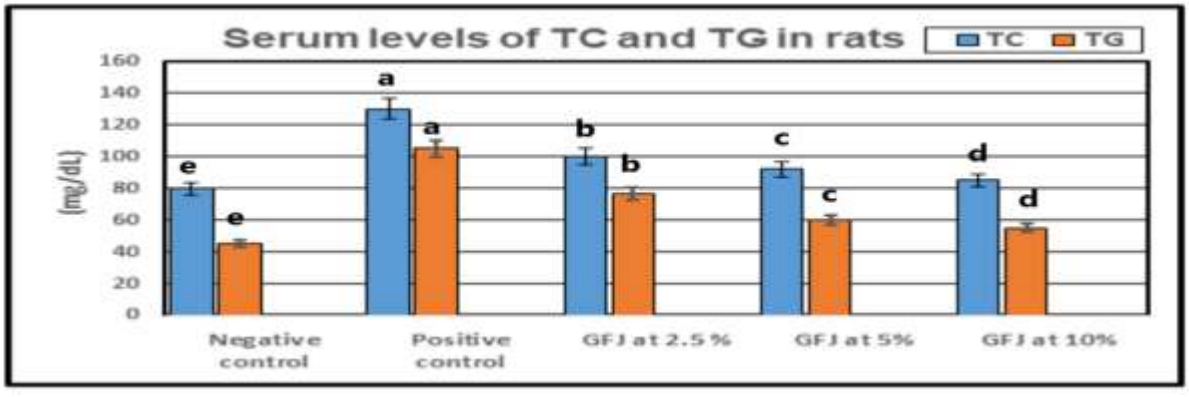

Fig. (1): Impact of grapefruit juice (GFJ) at 2.5., 5.0 and 10% on serum TC and TG levels within rats with ulcerative colitis.

Histopathology:

Fig. (2) illustrates the results of histopathological findings of rats' large intestines.

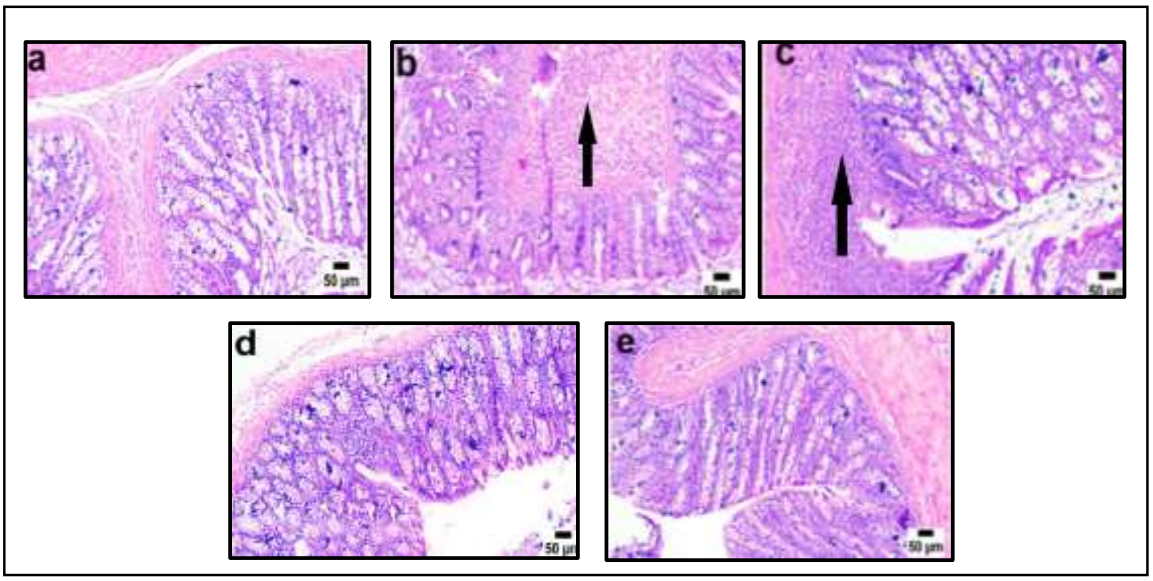

Fig. (2): Photomicrograph of rat colons from various experimental groups **:**

(a) Negative control group with a histologically normal colon's mucosa and sub-mucosa structure**.**

(b) Positive control rats with ulcerative colitis and orally given grapefruit juice at**%2.5** exhibiting lamina propria and sub-mucosa with heavy inflammatory cells infiltration**.**

(c) Rats given orally grapefruit juice at 2.5% displaying moderate focal area where inflammatory cell infiltrated the submucosal layer **.**

(d) Rats given orally grapefruit juice at 5.0 % displaying lamina propria with few foci of inflammatory cells aggregations**.**

(e) Rats given orally grapefruit juice at 10 % demonstrating apparent histologically normal architecture of colon mucosa and sub-mucosa.

The current research is intended to examine the protective impact of grapefruit juice (GFJ) at 2.5., 5.0, and 10% regarding acetic acid-induced UC in rats **.**

The present results indicated that grapefruit juice (GFJ) at 2.5, 5.0, and 10% significantly reduced body weight gain (BWG), feed efficiency ratio (FER), and feed intake (FI), resulting in weight reduction. This concur with the findings of Chudnovskiy et al. (2014). They determined that mice on a high-fat diet and grapefruit juice demonstrated a 18.4% reduction in body weight and a 13-17% decline in fasting blood glucose. Moreover, Alam et al. (2022) reported that naringin, common flavonoids present in GFJ, produced an anti-obesity effect**.**

The present data indicated that grapefruit juice (GFJ) at 2.5, 5.0, and 10% significantly decreased serum AST and ALT liver enzymes in UC rats. Our results coincide with Baleni et al. (2014), who documented that grapefruit juice and its bergamottin successfully inhibited the elevation of liver enzymes triggered by a paracetamol overdose, thus averting liver toxicity**.**

The current results showed that grapefruit juice (GFJ) at 10% significantly increased serum activities of antioxidant enzymes (GPx, SOD, and CAT). These results partly coincide with Zargar et al. (2018), who recognized that grapefruit juice considerably increased antioxidant enzymes activities (GPx, SOD, and CAT) in mice. The authors concluded that GFJ has elevated polyphenol compounds that could potentially enhance and act synergistically with the therapeutic effects of aripiprazole (antipsychotic drug). Castro-Vazquez et al. (2016) reported that grapefruit peel represents a valuable source of naturally occurring bioactive flavonoids with potent antioxidant effects, suggesting these compounds may serve as therapeutic agents that could be incorporated into multiple clinical approaches. Moreover, Alam et al. (2022) reported that naringin, common flavonoids present in GFJ, produced an antioxidant effect, anti-inflammatory, anti-atherosclerotic, hypochlosterolemic, hypolipidemic, neuroprotective, anti-diabetic, hepatoprotective, cardioprotective, and anti-obesity activities. In addition, broccoli extract produced anti-inflammatory and anti- oxidant effects during dextran sulphate sodium-induced UC within rats (Mueller et al. (2013**.(**

It was reported by Brody et al. (1996) that overproduction of reactive oxygen species (ROS) caused lipid peroxidation (LPO), which inhibited cellular anti-oxidant capacity that caused colon inflammation. Grisham and Yamada (1992) mentioned that inflammatory cytokines as TNFα, IL1 β, IL6 and IL8 are

recognized to significantly impact mucosal immune system, as the macrophages and neutrophils have the ability to compromise epithelial integrity and provoke colonic inflammation. Al-Rejaie et al. (2013) concluded that naringinin which is a common flavonoid in grapefruit juice produced anti-oxidant and anti-inflammatory effects, signifying a protective activity in IBD. Moreover, Castro-Vazquez, et al. (2016) mentioned that naringin and naringinin abundantly occur in grapefruit juice (GFJ) and contribute to its anti-oxidant and anti-inflammatory potentialities**.**

Our findings specified that, in rats with UC, the inflammatory effect is evidenced by the elevated proinflammatory cytokines levels, including TNF- α and interleukins. Oral administration of GFJ decreased serum inflammatory cytokines TNFα IL1 β, IL6 and IL8 in rats with UC. This finding agreed with Zargar et al. (2018), who reported that GFJ decreased serum inflammatory cytokines TNFα IL1 β, IL6 and IL8 in mice due to the anti-inflammatory and antioxidant effects**.**

Concerning serum total cholesterol (TC) and triglycerides (TG), our study exhibited that GFJ significantly lowered serum TC and TG levels. The obtained results agreed with Alam et al. (2022), who documented that naringin, a common flavonoid present in GFJ, produced hypochlosterolemic effect. In adults, Jonsson and Ellegard (2006) found that GFJ caused reduction in serum total cholesterol by 8% and LDL-c by 14%, but increased HDL-c by 6% in patients with hyperlipidemia. However, diet incorporating fresh GFJ induced a considerable reduction the serum lipid concentrations, particularly triglycerides. The inclusion of fresh GFJ in one's diet may provide benefits for patients with hyperlipidemia, particularly those with hypertriglyceridemia who are at risk of coronary atherosclerosis (Gorinstein et al. 2006).

4. Conclusion

It can be inferred that grapefruit juice at a concentration of 10% induces protection against ulcerative colitis because of its good anti-inflammatory and anti-oxidant effects by reducing inflammation and oxidative stress in rats. Therefore, consumption of grapefruit juice as a beverage could potentially offer benefits to individuals diagnosed with ulcerative colitis.

References:

1. Aebi, H.E. (1984): Catalase in vitro. Methods of Enzymol. ; 105: 121-126. Doi: 10.1016/s0076-6879(84)05016-3.
2. Alam, M.; Ahsan, F., Mahmood, T.; Shamim, A.; Parveen, S.; Shariq, M. and Ansari, V. A. (2022): Meticulous parade on naringin respecting its pharmacological activities and novel formulations. Avicenna J. Phytomed.; 12(5):25-30.
3. Al-Rejaie, S.S.; Aboulhashish, H.M.; Al-Nazi, M.M. Al Assaf, A.H.; Parmar, M.Y. and Ahmed, M.M. (2013): Protective effect of naringin on acetic acid induced ulcerative colitis in rats. World J. Gastroentrol.; 19 (34): 5633-5644 .
4. Allain, C.C.; Poon, L.S. and Chan, C.S. (1974): Enzymatic determination of serum total cholesterol. Clin. Chem.; 20: 470-475. Doi.org/10.1093/clinchem/20.4.470
5. Baleni, R.; Bakkar, Z. and Walubo, A. (2014): Grapefruit juice prevented the rise in liver enzymes after paracetamol overdose in rats. World Congress Pharmacol. ; 2014, At: Cape Town, South Africa. Doi: 10.13140/2.1.2585.2807
6. Balmus, I.M., Ciobica, A., Trifan, A. and Stanciu, C. (2016): The implications of oxidative stress and antioxidant therapies in inflammatory bowel disease: Clinical Aspects and Animal Models. Saudi J. Gastroenterol.; 22(1): 3-17. Doi: 10.4103/1319-3767.173753
7. Bancroft, J.D. and Stevens A. (1977): Theory and Practice of Histological Techniques. Edinburgh, Churchill-Livingstone, New York, Pages 740 .Doi.org/10.1111/j.1365-2559.1990.tb00755
8. Bergmeyer, H.U., Schreiber, P. and Wahlefeld, A.W. (1978): Optimization of methods for aspartate and alanine aminotransferases. Clin. Chem.; 24: 58-61 .PMID: 22409.
9. Brody, L.L.; Powell, S.R.; Collier, K.P.; Reddy and G.M. (1996): Increased oxidative stress and decreased antioxidant capacity in mucosa of inflammatory bowel disease. Dig. Dis. Sci.; 42: 2078-2086.
10. Cagin, Y.F.; Parlakpinar, H; Vardi. N.; Polat, A.; Atayan, Y. Erdogan, M.A. and Tanbek, K. (2016): Effects of dexpanthenol on acetic acid-induced colitis in rats. Exp. Ther. Med. ; 12(5): 2958-2964. Doi: 10.3892/etm.2016.3728
11. Castro-Vazquez, L.; Alañón, M.E.; Robledo, V.R. I.; Consuelo Díaz-Maroto, M.L;. Galindo, M.F. and Arroyo Jiménez, M. (2016): Bioactive Flavonoids, Antioxidant Behavior, and Cytoprotective Effects of Dried Grapefruit Peels . Oxid. Med. Cell Longev.; 2016: 8915729. Doi: 10.1155/2016/8915729
12. Chapman, D. G., Gastilla, R. and Campbell, J. A. (1959): Evaluation of protein in foods: 1- A Method for the determination of protein efficiency ratio. Can. J . Biochem. Phys.; 37:679-686. Doi.org/10.1139/o59-074
13. Chudnovskiy, R.; Thompson. A.; Tharp, K.; Hellerstein, M.; Napoli, J.L. and Stahl. A. (2014): Consumption of Clarified Grapefruit Juice Ameliorates High-Fat Diet Induced Insulin Resistance and Weight Gain in Mice. PLoS One.; 9(10): e108408.

14. Golechha, M.; Chaudhry, U.; Bhatia, J.; Saluja, D.; Arya, D.S. (2011): Naringin protects against kainic acid-induced status epilepticus rats: evidence for an antioxidant, anti-inflammatory and neuroprotective intervention. Biol. Pharm .Bull.; 34: 360-365.
15. Gorinstein, S.; Caspi, A.; Libman, I.; Lerner, H.T.; Huang, D.; Leontowicz, H ;. Leontowicz, M.; Tashma, Z. and Katrich, E. (2006): Red grapefruit positively influences serum triglyceride level in patients suffering from coronary atherosclerosis: studies in vitro and in humans. J. Agric. Food Chem.; 54(5):1887-1892 .Doi: 10.1021/jf058171g
16. Grisham, M.B. and Yamada, T. (1992): Neutrophils, nitrogen oxides and inflammatory bowel diseases. Ann. NY Acad. Sci.; 664:103-115.
17. Fukuda, K.; Ohta, T. and Yamazoe, Y. (1997): Grapefruit component interacting with rat and human P450 CYP3A: possible involvement of non-flavonoid components in drug interaction. Biol. Pharm. Bull.; 20 (5):560-564 .Doi: 10.1248/bpb.20.560.
18. Hwang, S. L.; Shih P. H. and Yen, G. C. Neuroprotective effects of citrus flavonoids :(2012) .J. Agric. Food Chem.; 60(4):877-885. Doi: 10.1021/jf204452y
19. Jollow, D.J., Thorgeirsson, S.S., Potter, W.Z. Hashimoto, M. and Mitchell, J.R. (1974): Acetaminophen-induced hepatic necrosis.VI. Metabolic disposition of toxic and nontoxic doses of acetaminophen. Pharmacol.; 12(4-5):251-271 .Doi: 10.1159/000136547.
20. Jonsson, C. and Ellegard, L. (2006): Grapefruit juice and serum lipids in healthy adults. Scandinavian J. Food Nutri. ; 50(3):118-123. Doi: 10.1080/17482970600967504
21. Khor, B.; Gardet, A. and Xavier. R.J. (2011): Genetics and pathogenesis of inflammatory bowel disease. Nature; 474:307-317. Doi: 10.1038/nature10209 .
22. Magro, F. (2017): Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 1: definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and Ileo-anal pouch disorders. J. Crohns and Colitis; 11, 649-670. Doi: 10.1093/ecco-jcc/jjx008.
23. Mendes, C.; Rocha, J.; Direito, R.; Fernandes A.; Sepodes B.; Maria-Eduardo, F.; Maria, H. and Ribeiro, M. (2019): Anti-inflammatory activity of grapefruit juice in an in vivo model of ulcerative colitis: Comparability studies of unprocessed and bioprocessed juices. J. Function. Foods.; 63: 103564
24. Mueller, K., Blum, N.M. and Andreas Stefan Mueller, A.S. (2013): Examination of anti-inflammatory, antioxidant and xenobiotics-inducing potential of Broccoli extract during DSS-induced colitis in rats. Inter. Schol. Res. Notes; 2013 | Article ID 710856. Doi.org/10.1155/2013/710856
25. Ng, S.C., Bernstein, C.N., Vatn, M., Lakatos, P. and Tysk, C. (2013): Geographical variability and environmental risk factors in inflammatory bowel disease. Gut. ; 62(4): 630-649. Doi.org/10.1136/gutjnl-2012-303661
26. Nishikimi, M.; Appaji, N. and Yagi, K. (1972): The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. Biochem. Biophys. Res. Commun. ; 46(2): 849-854.Doi.org/10.1016/S0006-291X (72)80218-3
27. Ohkawa, H., Ohishi, N. and Yagi, K. (1979): Assay for lipid peroxides in animal Tissues by thiobarbituric acid reaction (TBA). Analyt. Biochem. ; 95:351- 358 .
28. Racz, O.; Nistiar, F.; Sipulova, A. and Riemerova, M. (1994): Glutathione peroxidase in blood of diabetic and nondiabetic BB rats. Arch. Gerontol Geriat.; 19(1):207-216.Doi.org/10.1016/S0167-4943 (05)80066-4
29. Reeves, P.G.; Nielson, F.H. and Fahmy, G.C. (1993): Reports of the American Institute I of Nutrition, ADHOC Willing Committee on Reformulation of the AIN 93, Rodent diet. J. Nutri.; 123(1):1939-1951. .Doi: 10.1093/jn/123.11.1939
30. Scaldaferri, F. and Fiocchi, C. (2007): Inflammatory bowel disease: progress and current concepts of etiopathogenesis. J. Dig. Dis.; 8:171-178
31. Sinha, A.K. (1972): Colorimetric assay of catalase. Analyt. Biochem. ; 47, (2), 389-394 .Doi: 10.1016/0003-2697(72)90132-7.
32. Snedecor, G.W. and Cochran W.G. (1986) "Statistical Methods". 6th Ed. Iowa State University Press, Ames, Iowa, USA. pp 381-418.
33. Sutton, C.; Brereton, C. Keogh, B. Kingston, H.G.M. and Ed C. Lavelle (2006): A crucial role for interleukin (IL)-1 in the induction of IL-17–producing T cells that mediate autoimmune encephalomyelitis .J. Exp. Med.; 203(7):1685-1691. Doi: 10.1084/jem.20060285
34. Wahlefeld, A.W., Triglycerides determination after enzymatic hydrolysis, In: Methods of Enzymatic Analysis. Ed. H. U. Bergmeyer, 2nd English ed. Academic Press, New York (USA) 1974; pp. 18-31.
35. Wang, G., Xu, B., Shi, F., Du, M., Li, Y., Yu, T. and Chen, C. (2019):Protective effect of methane-rich saline on acetic acid-induced ulcerative colitis via blocking the TLR4/NF-κB/MAPK pathway and promoting IL-10/JAK1/STAT3- mediated anti-inflammatory response. Oxid. Med. Cell. Longev. ; 2019 2019:7850324 .Doi: 10.1155/2019/7850324
36. Xia, B., Crusius, J.B.A., Meuwissen. S.G.M., and Peña, A.S. (1998): Inflammatory bowel disease: definition, epidemiology, etiologic aspects, and immunogenic studies. World J. Gastroenterol.; 4(5): 446-458.Doi: 10.3748/wjg.v4.i5.446
37. Zargar, S.; Al-Majed, A.A. and Wani, T.A. (2018): Potentiating and synergistic effect of grapefruit juice on the antioxidant and anti-inflammatory activity of aripiprazole against hydrogen peroxide induced oxidative stress in mice. BMC Complement. Altern. Med.; 18, Article number: 10