

Evaluation of the combined effects of Turkish mad honey and 5-Fluorouracil in colon cancer model in rats

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ABSTRACT

It was aimed to evaluate the regressive effect of grayanotoxin-rich Turkish mad honey and 5-fluorouracil (5-FU), separately and together by using the N-methyl-N-nitrosourea (MNU)-induced colon cancer modelling in rats. Study groups were designed as control group (CG), cancer control group (CCG), 5-Fluorouracil group (FUG), Turkish mad honey group (HG), Turkish mad honey and 5-FU combined group (FU-HG). White blood cell (WBC), lymphocyte, eosinophil, basophil, serum lactate dehydrogenase (LDH), total oxidant status (TOS), and total protein values of the rats in the CCG were significantly lower than the values of the rats in the CG, whereas serum Bcl-2 and survivin levels were significantly higher in the rates belonged to the CCG in comparison to those in the CG. The presence of anaplastic epithelial cells, vascularization, precancerous changes, and inflammatory infiltration detected in the colon and small intestine of the rats in FU-HG, FUG, HG were less intense ($P < 0.05$) compared to the findings in the rats in CCG. In conclusion, mad honey and 5-FU reduced anaplastic cell growth and oxidative stress via suppressed anti-apoptotic activity. Considering the histopathological findings in the liver and kidney, no toxicity occurred related to mad honey and 5-FU metabolism. Therefore, the combined use of these two substances may be an alternative method in the treatment of colon cancer.

Introduction

The histopathologic changes in the colorectal cancer (CRC) consist of precancerous changes such as the formation of abnormal crypt foci and mucosal cell aggregations, as well as cellular changes ranging from polyps and adenomas to adenocarcinomas (20).

5-Fluorouracil (5-FU) is an effective and frequently used therapeutic agent in the developmental stages of CRC, when it acquires malignancy characteristics (10). It is suggested that the use of specific antioxidants together with chemotherapeutics in cancer treatment can reduce the incidence of side effects related to cancer drugs and increase the anti-carcinogenic effects of these drugs (19). Antioxidants can effectively protect cells against damage, and therefore they are of vital importance for the

homeostasis of cells and tissues (5, 25). A large number of phenolic compounds in the content of Turkish mad honey give the honey its antioxidant properties. These substances defend the cells against the attacks of free radicals and inhibit metabolic action processes (15, 16, 30).

In the CRC model ted with MNU, a directly proportional relationship was determined between inflammation and degenerative changes in the colonic mucosa and oxidative stress (5, 16). Also the oxidative stress index (OSI) value expressed by the total oxidant status (TOS)/total antioxidant status (TAS) ratio is an important parameter in the evaluation of oxidative stress (37). In the evaluation of oxidative stress with PON-1, which is an indicator of oxidation in tissues and PON-1 levels changes due to the changes in the redox status (6, 10).

In order to increase the quality of life in cancer patients, it is very important to investigate the effects of antioxidant substances contained in mad honey against the chemotherapeutic activity and side effects of 5-FU (1, 34). Also the antioxidant properties and cytotoxic activities of Turkish mad honey and *Rhododendron ponticum* extracts have been evaluated in many studies *in vivo* and *in vitro* (7-9, 14, 29, 39).

For this purpose, it was aimed to evaluate the effects of 5-FU and Turkish mad honey, which are used in the treatment of experimental CRC induced by MNU, on the histopathological changes, oxidative stress and apoptotic mechanisms in cancerous tissues.

Materials and Methods

This study was approved by the Ankara University Animal Experiments Local Ethics Committee (Approval no: 2020-7-55). In the study, 6 weeks old (average weight of 150–180 g), 30 male Wistar albino rats were used. They were given standard feed and water ad libitum throughout the study and maintained an air-conditioned animal facility under constant temperature and humidity with a 12 h day-night cycle. The first group of 6 rats constituted the control group (CG), which did not receive any treatment. The solution, obtained by dissolving 2 mg/rat N- MNU in 0.5 mL of water, was administered rectally to the remaining 24 rats 3 times a week for 5 weeks to induce colon cancer. These 24 rats were then randomly divided into 4 groups (n=6/each group). The second group, consisting of 6 rats, was the cancer control group (CCG), which did not receive any treatment. The third group, consisting of 6 rats, constituted the honey group (HG), in which Turkish mad honey was administered orally at a dose of 0.3 mg/g BW 3 times a week for 4 weeks. The fourth group, consisting of 6 rats, constituted the 5-FU group (FUG), in which 5-FU was administered intraperitoneally at a dose of 12.5 mg/kg BW 3 times a week for 4 weeks. The fifth group consisted of 6 rats (FU-HG) in which 12.5 mg/kg BW of 5-FU was administered 3 times a week for 4 weeks and 0.3 mg/g BW of Turkish mad honey 3 times a day was administered orally by gavage for 4 weeks.

Sampling: Honey samples collected from the Black Sea region in Türkiye and brought in glass jars right after the honey harvest were stored at -80°C until the study was conducted. Before the study, the honey samples were taken out of the freezer and left until they reached room temperature, and these samples were then diluted 1 to 3 with deionized water. In order to provide a good mixture, the mixing process was carried out in a vortex (Heidolph, Reax 2000) device and made ready for study (32).

A total of 7 mL of blood, 5 mL for each tube without anticoagulant and 2 mL for each tube with EDTA, was collected from the rats. Serum samples were removed

within 3 hours of the collection of blood samples and these serum samples were stored at -80 °C until analysis.

Blood Analysis: The hematological analyses were performed using an automatic blood count device (Mindray BC 5000) within 3 hours of blood collection. In the serum samples, CRP, total protein, albumin, LDH, ALT, AST, urea, and creatinine levels were measured with an automated biochemistry analyzer (Mindray BS300). Serum Bcl-2, epidermal growth factor, colon cancer specific antigen, matrix metalloproteinase-7, and survivin levels were measured spectrophotometrically, using the ELISA method and related ELISA kits (Sun Red Biotechnology Company, No: 201-11-0038 for Bcl2, No: 201-11-0153 for epidermal growth factor, No: 201-11-6117 for colon cancer-specific antigen-3, No: 201-11-0320 for matrix metalloproteinase-7, and No: 201-11-0192 for survivin).

Histopathologic Analysis: After the rats were sacrificed, necropsy was performed. Possible cancer developing areas and pathological changes in the liver and kidneys as well as other tissues and organs were evaluated according to general macroscopic definition criteria. Samples were fixed in 10% formalin solution for 48 hours. Then, for histopathological examination, the tissues were followed-up at ethanol series and xylene in an automatic vacuum tissue processor (TP1020, Leica, Germany) and embedded in paraffin in a paraffin dispenser (Leica, EG1150H, Germany). Next 5- μ m-thick sections were taken from the paraffin blocks (Shandon AS320). All tissue sections were stained according to the standart hematoxylin–eosin (H&E) staining method. Sections were covered with the cover slip using the mounting medium Entellan® (Merck). Tissue sections were evaluated semiquantitatively by counting 10 fields under a digital light microscope (Olympus BX51) at 400 \times magnification. Malignant colonic epitheliums and gland cells as well as proportions in tissue distribution were taken into attention. The results of the evaluations scored according to their numerical equivalents were scores from 1 to 6 (1 =0-4%; 2=5-19%; 3 = 20-39%; 4 = 40-59%; 5 = 60-79%; 6 = 80-100%). The scoring were done on the basis of intensity of nuclear hyperchromasia in malignant cells and proportion as proposed in Quickscore (QS) method modified by Detre et al. 1995 (12). Differences in the numbers of the groups were evaluated statistically. Eligible sites chosen under microscopy were visualized.

Statistical Analysis: The data were initially summarized with descriptive statistics and checked for whether assumptions were met. Results were evaluated by using Shapiro-Wilk test and Q-Q plot for normality and the Levene test for homogeneity of variances. The WBC, lymphocyte, CRP, total protein, LDH, AST, Urea,

Albumin, rat EGF elisa kit, Rat bcl-2 elisa kit, PON-1 and MMP-7 parameters were found to be normally and heteroscedasticity distributed thus One-Way Anova was used. Monocyte, Creatinine, Rat CCAG3 elisa kit, Rat Survivin elisa kit, TAS parameters' variances were not homogen, consequently, Welch's ANOVA was used. Because of violating both normal and heteroscedasticity distribution, neutrofil, ezonofil, basophil, ALT, TOS and OSI parameters were compared with Kruskal-Wallis test. Furthermore, histopathological examinations were compared using and One-way ANOVA (GraphPad, USA). In case of statistically significant difference between groups, Tukey, Games-Howell or DSCF multiple comparison analysis were used appropriately. The data were analysed using IBM SPSS Statistics 26.0 (SPSS®, IL, USA) statistical software. The results were analyzed and expressed as mean \pm standard error (M \pm SE). A P value of <0.05 was considered to be statistically significant for all analyses.

Results

Hematological Results: The WBC, lymphocyte, monocyte, neutrophil, eosinophil, and basophil values of

the rats in the CCG, FU-HG, FUG, HG, and CG groups are shown in Table 1.

Biochemical Findings: The blood serum CRP, total protein, albumin, LDH, ALT, AST, urea, and creatinine values of the rats in the CCG, FU-HG, FUG, HG, and CG are shown in Table 1.

The blood serum CCSAG3, TAS, TOS, OSI, and PON-1 and tissue EGF, survivin, Bcl-2, MMP-7, values of the rats in the CCG, FU-HG, FUG, HG, and CG groups are shown in Table 1.

Histopathological Findings: Small intestine and colon: Anaplastic glandular and crypt epitheliums were evaded into propria mucosa. While capillarization and hyperemia in the capillaries and medium-sized vessels were observed in the large intestine, these changes were not observed in the small intestine. In both the small intestine and colon, inflammatory changes, usually moderate, in the form of mononuclear cell infiltrations were detected. There were precancerous areas, more intense in the CCG. In these areas, more proliferating dysplastic cells were observed. These cells did not meet the anaplastic criteria and were similar to each other in terms of uniformity.

Table 1. Hematologic-Biochemical parameters according to experimental groups.

Groups	Hematologic-Biochemical Parameters					P
	CCG (n=6)	FU-HG (n=5)	FUG (n=6)	HG (n=6)	CG (n=6)	
WBC (10 ⁹ /L)	5.84 \pm 0.62 ^b	8.89 \pm 0.32 ^a	5.97 \pm 0.6 ^b	6.96 \pm 0.25 ^{ab}	8.19 \pm 0.31 ^a	P<0.001
Lymphocyte (10 ⁹ /L)	4.02 \pm 0.44 ^b	6.49 \pm 0.42 ^a	4.42 \pm 0.41 ^b	5.08 \pm 0.2 ^{ab}	6.04 \pm 0.24 ^a	P<0.001
Monocyte (10 ⁹ /L)	0.36 \pm 0.04 ^a	0.38 \pm 0.04 ^a	0.32 \pm 0.03 ^{ab}	0.24 \pm 0.02 ^b	0.33 \pm 0.06 ^{ab}	P=0.028
Neutrophil(10 ⁹ /L)	1.36 \pm 0.22 ^{ab}	1.88 \pm 0.09 ^a	1.28 \pm 0.16 ^b	1.46 \pm 0.1 ^a	1.57 \pm 0.08 ^a	P=0.049
Eosinophil (10 ⁹ /L)	0.07 \pm 0 ^b	0.1 \pm 0 ^{ab}	0.08 \pm 0.01 ^b	0.15 \pm 0.01 ^a	0.18 \pm 0.04 ^a	P<0.001
Basophil (10 ⁹ /L)	0.03 \pm 0 ^b	0.05 \pm 0 ^b	0.11 \pm 0.06 ^a	0.03 \pm 0 ^b	0.18 \pm 0.04 ^a	P=0.003
CRP (mg/L)	0.45 \pm 0.02 ^a	0.22 \pm 0.06 ^b	0.35 \pm 0.04 ^{ab}	0.43 \pm 0.04 ^a	0.05 \pm 0 ^b	P=0.008
TP (g/dL)	6.01 \pm 0.17 ^a	7.69 \pm 0.44 ^{ab}	7.48 \pm 0.54 ^{ab}	7.93 \pm 0.46 ^a	0.11 \pm 0.06 ^a	P=0.018
Alb (g/dL)	3.91 \pm 0.09	3.86 \pm 0.18	4.11 \pm 0.21	4.42 \pm 0.22	0.03 \pm 0 ^b	
LDH (U/L)	2447 \pm 225.06 ^c	3418 \pm 371.29 ^{bc}	2940.33 \pm 433.24 ^{bc}	5868.83 \pm 252.72 ^a	0.07 \pm 0.01 ^a	P<0.001
ALT(U/L)	100 \pm 7.63	65.4 \pm 6.3	143 \pm 47.87	74.67 \pm 5.96	86.83 \pm 7.89	
AST(U/L)	180.33 \pm 16.86	183.4 \pm 27.19	185.67 \pm 13.04	241 \pm 20.57	201 \pm 8.61	
Urea (mg/dL)	60.67 \pm 2.43	58.4 \pm 2.4	63.83 \pm 3.15	63.83 \pm 1.9	66.17 \pm 2.76	
Creatinine (mg/dL)	1.26 \pm 0.05 ^a	0.94 \pm 0.03 ^b	1.11 \pm 0.11 ^{ab}	1.08 \pm 0.05 ^{ab}	1.11 \pm 0.05 ^{ab}	P=0.003
CCSAG3 (ng/mL)	90.06 \pm 2.92	89.46 \pm 7.72	86.85 \pm 3.05	86.02 \pm 5.27	82.08 \pm 6.2	
EGF (ng/L)	90.75 \pm 1.24	60.84 \pm 5	66.89 \pm 2.29	66.63 \pm 1.95	66.46 \pm 4.79	
Survivin (pg/mL)	58.75 \pm 4.05 ^a	17.6 \pm 1.42 ^b	15.01 \pm 1.83 ^b	15.55 \pm 2.2 ^b	7.43 \pm 0.44 ^b	P<0.001
Bcl-2 (ng/mL)	18.47 \pm 0.69 ^a	12.15 \pm 0.79 ^b	13.83 \pm 0.72 ^b	12.32 \pm 1.43 ^b	12.88 \pm 1.17 ^b	P<0.001
MMP-7 (μ g/L)	0.22 \pm 0.02	0.17 \pm 0.01	0.18 \pm 0.01	0.2 \pm 0.02	0.15 \pm 0.01	
TAS (mmol/L)	1.53 \pm 0.1	1.66 \pm 0.11	2.19 \pm 0.38	1.91 \pm 0.11	1.89 \pm 0.13	
TOS (μ mol/L)	2.78 \pm 0.54 ^b	17.68 \pm 2.94 ^a	14.08 \pm 1.47 ^a	19.61 \pm 1.59 ^a	18.79 \pm 2.56 ^a	P=0.002
OSI	1.77 \pm 0.62 ^b	10.93 \pm 4.56 ^a	7.66 \pm 3.96 ^{ab}	10.72 \pm 3.91 ^a	10.11 \pm 3.47 ^a	P=<0.001
PON-1 (U/L)	488.17 \pm 32.01 ^{bc}	220.6 \pm 32.7 ^c	446.5 \pm 70.04 ^c	884 \pm 63.59 ^a	722.67 \pm 99.77 ^{ab}	P<0.001

Values in the table are given as arithmetic mean \pm standard deviation (M \pm SD).

*a,b indicate differences between groups in the same column.

Atypic cells in the small intestine in the CCG animals were statistically significantly higher than the values determined in the rats in the CG (respectively $P=0.049$, $P=0.014$, $P=0.016$, $P=0.014$, $P=0.012$, $P=0.015$, $P=0.023$). In addition, a statistically significant decrease was determined malignancy, vascularization, and precancerous images seen in the FU-HG, unlike the findings in the CCG (respectively $P=0.049$, $P=0.014$, $P=0.043$, $P=0.024$, $P=0.012$, $P=0.015$). The aforementioned findings determined in the HG were significantly different to the findings in the CCG ($P=0.045$, $P=0.012$, $P=0.015$, respectively). In addition, a statistically significant increase was found in the pleomorphism and inflammatory infiltration levels determined in the HG rats, compared to the CG rats (respectively $P=0.015$, $P=0.019$). It was noted that the inflammatory infiltration status determined in the rats in the FUG was different from the findings in the HG, CG, and CCG rats ($P=0.031$, $P=0.023$, $P=0.023$, respectively).

The previous findings as well as inflammatory infiltration values detected in the colons of rats in the CCG showed a statistically significant increase compared to the values determined in the rats in the CG (respectively $P=0.015$, $P=0.014$, $P=0.014$, $P=0.012$, $P=0.008$, $P=0.015$, $P=0.016$). In addition, the malignancy findings in the FU-HG were also statistically significantly lower than the findings in the rats in the CCG ($P=0.002$, $P=0.014$, $P=0.023$, $P=0.022$, $P=0.008$, $P=0.015$, respectively). Such findings in the HG and FUG rats were significantly decreased compared to the data in the CCG rats ($P=0.048$, $P=0.045$, $P=0.008$, $P=0.015$, respectively). The inflammatory infiltration values determined in the HG, FUG, and FU-HG rats were significantly different from those determined in the CG rats ($P=0.014$, $P=0.014$, $P=0.017$, respectively).

Liver: Areas of predominantly vacuolar degeneration and less necrosis started at the periphery of the central vein parts of the lobes and increased at the periphery at varying degrees according to the groups. The vessels and sinusoids in the central vein and portal regions were filled with erythrocytes at different densities. There was no inflammatory cell infiltration as a reaction. Kupffer cells were hypertrophic and hyperplastic.

A significant difference was found between the CG and CCG rats in terms of degeneration, vascular changes, and increases in Kupffer cell activation ($P=0.012$, $P=0.012$, $P=0.008$, respectively). The degeneration detected in the FU-HG, FUG, and HG rats was significantly less severe than that in the CCG rats ($P=0.012$, $P=0.03$, $P=0.019$, respectively). A significant increase was found in the vascularization determined in the rats in the CCG, compared to the results of the evaluations made in the other groups of rats ($P=0.016$, $P=0.028$, $P=0.015$, respectively).

Kidney: Areas showing degeneration were observed in the tubular epithelium of the cortical and medullary regions,

especially in the cortical tubular epithelial cells, with varying degrees according to the groups. Tubular degeneration and hypercellularized glomeruli as well as hyperemia in capillaries and medium-sized vessels were noted according to the groups.

Significant increases were found in the data for degeneration, vascular changes, and mesangial cell and podocyte activation in the CCG rats compared to the data in the CG rats ($P=0.012$, $P=0.012$, $P=0.008$, respectively). In the evaluations made in terms of degeneration, the data determined in the HG, FUG, and FU-HG rats were significantly lower than the value in the CCG rats ($P=0.012$, $P=0.021$, $P=0.012$, respectively). The changes in the tissues are shown in Fig. 1-5.

Discussion and Conclusion

The main reason for the frequent occurrence of cancer in the gastrointestinal tract is the occurrence of cell signal pathways that change depending on genetic and environmental factors in the cells in this region (11, 24, 28).

It has been reported that there is a direct correlation between the oxidative stress and inflammatory response in the colon and small intestinal tissue after MNU application and the severity of carcinogenesis (28). Akcilar et al. (3) examined ischemia-reperfusion (IR) damage in the rat intestine, and they determined an increase in serum TAS level and a decrease in TOS level. On the other hand, Dogan et al. (13) reported that there were no significant differences between the groups in terms of serum TOS and OSI levels in IR model rats. Jisha et al. (26) reported a positive correlation between the severity of oxidative stress and inflammation and the developmental rate and severity of the pathogenesis of the tumor in the colon cancer model. In addition, they found significant differences in the serum CRP levels between the studied groups. In our study, we interpreted the fact that the serum CRP value determined in the FU-HG rats was significantly lower than the value determined in the CCG rats as evidence of the anti-inflammatory effect of the combination of 5-FU and mad honey. In addition, we found that serum TOS and OSI value determined in the CCG rats was significantly lower than the values in the other groups. We attributed the low serum TOS and OSI values to the occurrence of antiapoptotic and proliferative activities metabolites. We also thought that the molecular mechanism and pathways could demonstrate the exact mechanism. The fact that the serum PON-1 value determined in the HGG rats was significantly lower than the value determined in the CCG rats, was attributed to the anti-inflammatory and antioxidative effects of the bioactive substances in Turkish mad honey. Ahmed et al. (2) stated that mad honey, which contains bioactive substances such as polyphenols and flavonoids with strong antioxidant properties, is used to prevent and eliminate pathological changes in the body due to its free radical scavenging effect.

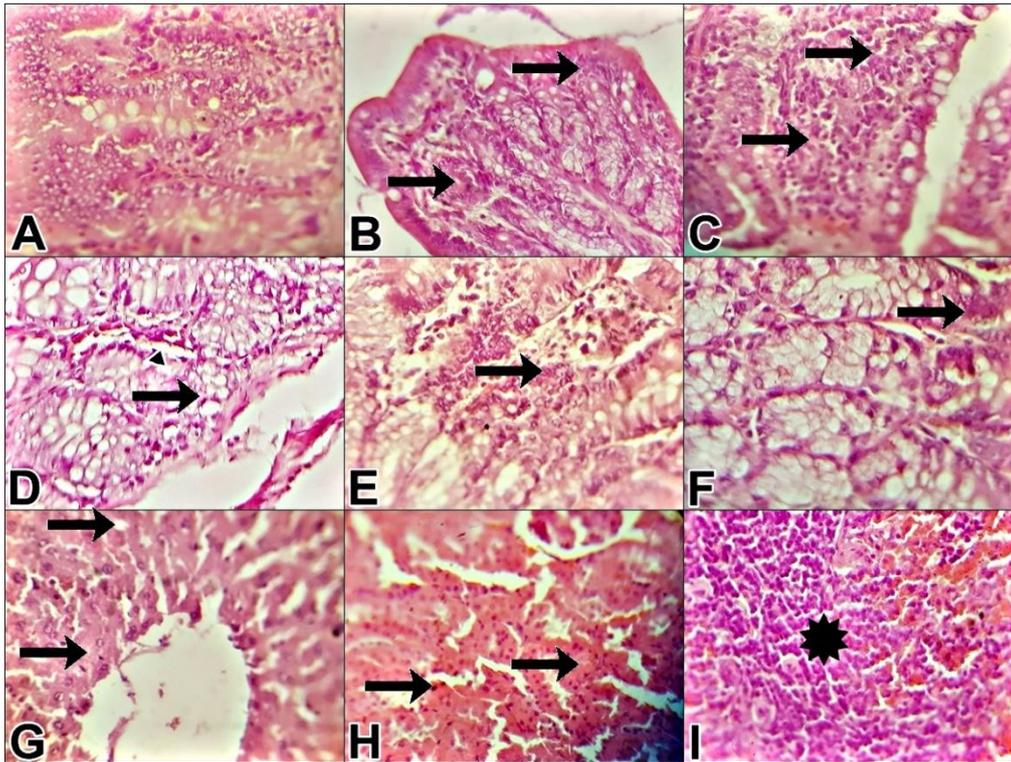


Figure 1. Histopathological findings in the CCG.

(A) Precancerous and cancerous changes in glands in the lamina propria of the intestine, x100, (B) Proliferation of atypical glandular epithelial cells (arrows), x200, (C) Mononuclear cell infiltrations (arrows) in the lamina propria, x400, (D-E) Proliferation of atypical cells in the colon gland epithelium (arrow) and vascularization (arrowhead) in the interstitium, x200, and (F), x400, (G) Congestion in the central vein in the liver and vacuolar degeneration (arrows) in hepatocytes (G), x200, (H) Acute cell swelling and several vacuolar degenerations (arrows) in cortical tubular epithelium of the kidney, (H), x200, (I) Hyperplastic lymphoid follicle in spleen (star), x400, H&E staining.

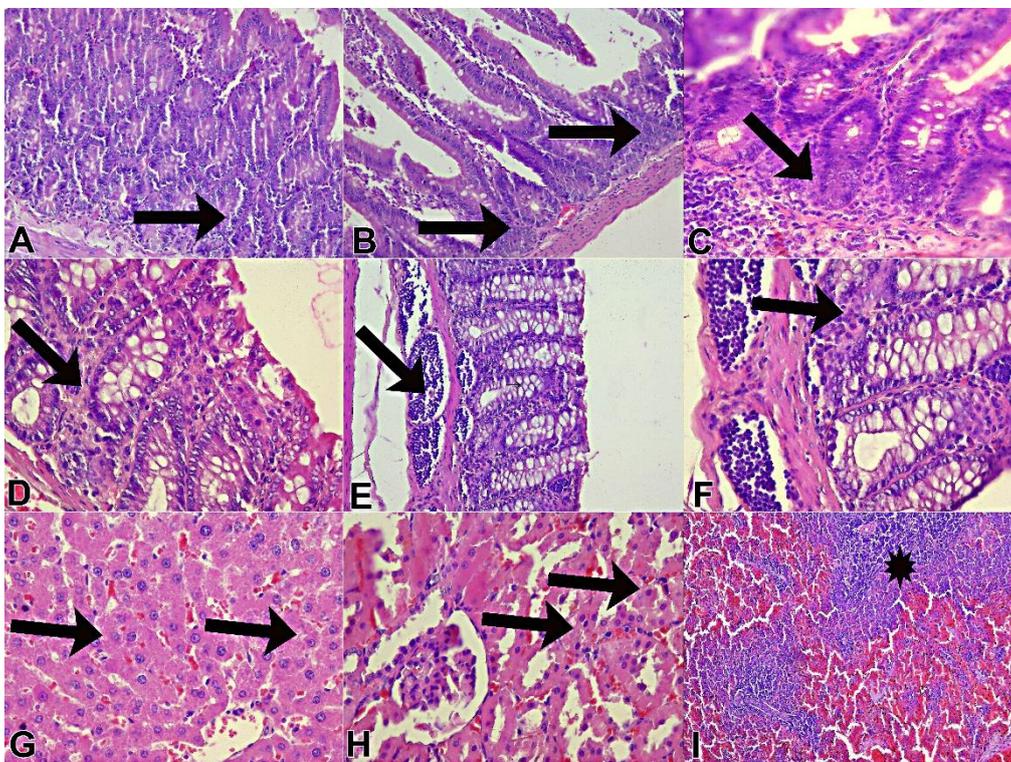


Figure 2. Histopathological findings in the HG.

(A) Atypical proliferation (arrow) in intestinal crypt and glandular epitheliums, x40, (B) Atypical proliferation in glandular epithelial cells (arrows), x40, Mononuclear cell infiltrations in the lamina propria (arrow), x100 and (C), x200, (D-E) Atypical cells in the colon gland epithelium proliferation and mononuclear cell infiltrations (arrow) in the lamina propria, x100, and (F), x200, (G) Congestion of sinusoids in the liver, vacuolar degeneration (arrow) in a few hepatocytes, x200, (H) Congestion of interstitial vessels and acute cell swelling in a few cortical tubular epithels (arrows) in the kidney, x200 (I) Hyperplastic lymphoid follicle (star) in spleen, x40, H&E staining.

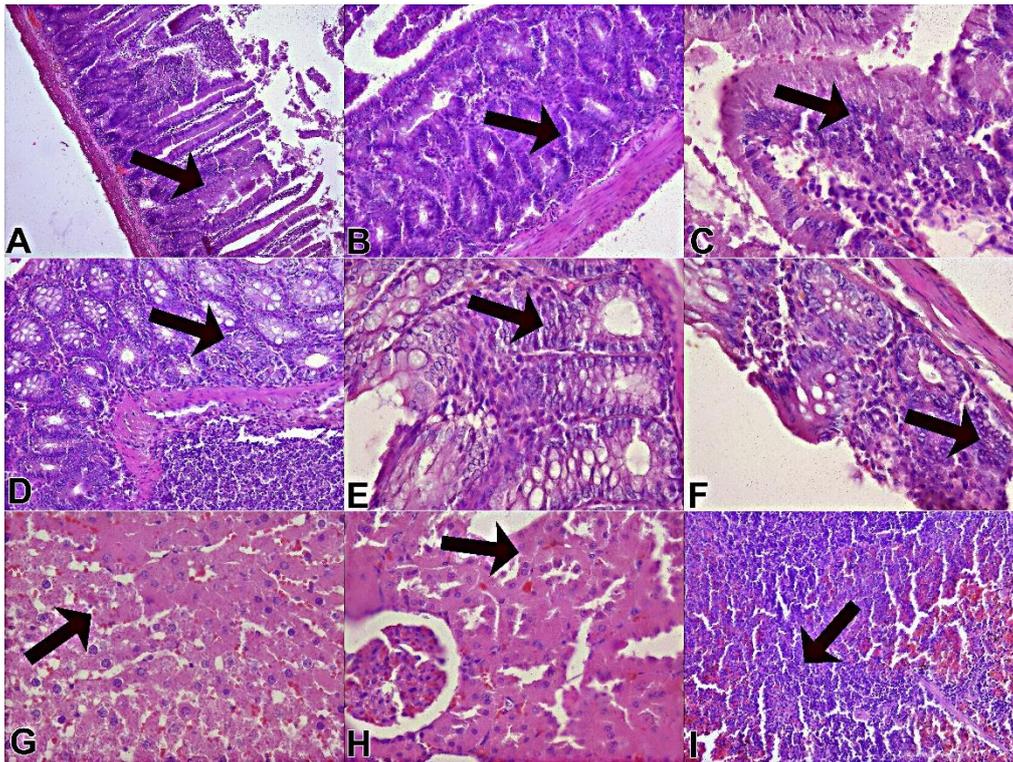


Figure 3. Histopathological findings in the FUG.

(A) Atypical proliferation in intestinal crypt and glandular epitheliums, x40, (B) Atypical proliferation in glandular epithelial cells (arrows), x100, (C) Proliferation in epithelial cell (arrow) and mononuclear cell infiltrations in the lamina propria, x200, (D-F) Proliferation of atypical cells in glandular epithelium (arrow) and mononuclear cell infiltrations in lamina propria of colon x40 and x100. (G) Vacuolar degeneration of hepatocytes (arrow) in liver, x200. (H) Acute cell swelling and several vacuolar degenerations (arrow) in epithelial cells of cortical tubules (arrow), x200, (I) Hyperplastic change in lymphoid follicle in spleen (arrow), x200, H&E staining.

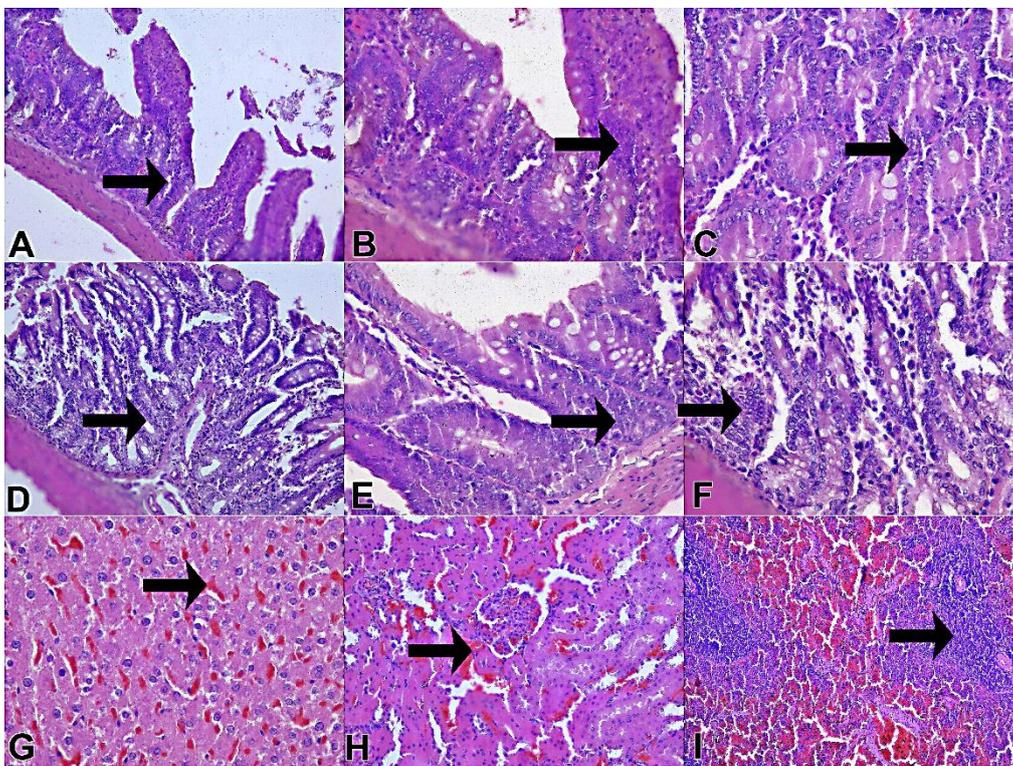


Figure 4. Histopathological findings in the FU-HG.

(A) Proliferation of atypical cells (arrow) in intestine, x40, (B-C), x200, (D-E) Proliferation of atypical cells in a few areas in glandular epithelium (arrow) in colon, x100, and (F), x200, (G) Congestion in sinusoids (arrow) in liver, x200, (H) Interstitial vessels (arrow) in kidney congestion, (H), 200×, (I) Lymphoid follicles (arrow) in spleen, x40, H&E staining.

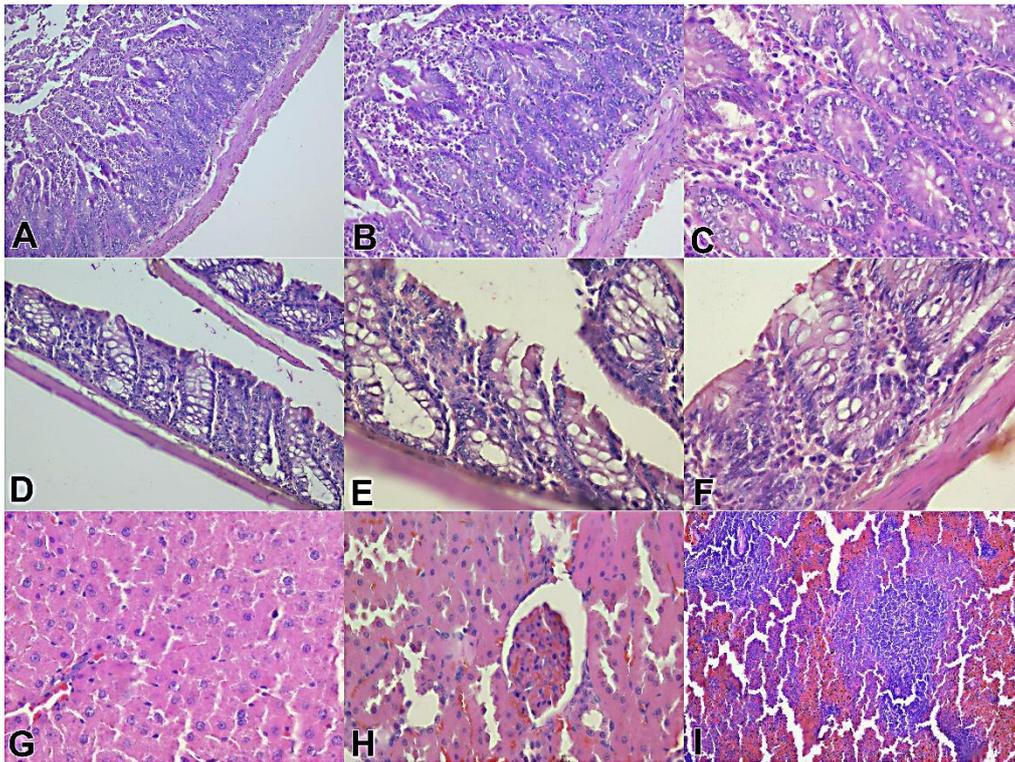


Figure 5. Histopathological views in the CG.

No finding, (A-B) Intestines, x40, and (C), x200, (D) Colon epithelium x40, (E-F), x100, (E-F), x200, (G) Liver, x200, (H) Kidney, x200, (I) Spleen, x40, H&E staining.

In studies conducted in the colon cancer model created with MNU, serum CCSA-3, anti-proliferative effect (TGF, EGF), pro-apoptotic survivin, Bcl2, anti-metastasis MMP-7, and anti-inflammatory CRP levels were evaluated (17, 18, 35). Iliemene and Atawodi (23), determined that the administration of *Brachystegia eurycoma* (Harms) was effective against serum CEA level, oxidative stress, and inflammatory responses, and ultimately procarcinogenic formations. Rehman et al. (33), determined that piperine application had positive effects on CEA, oxidative stress, and inflammation markers. Hamza et al. (19) attributed the decrease in serum transforming growth factor-beta (TGF- β), carcinoembryonic antigen (CEA), matrix metalloproteinase-7 (MMP-7), and colon cancer specific antigen-4 (CCSA-4) levels to the curative effects of fluorouracil and turmeric applications against inflammation, proliferation, and apoptosis in a colon cancer model. Ahmed et al. (2) determined that *Punica granatum* caused a decrease in CAE and CCSA levels (which are colon cancer markers), TGF- β and EGF levels (which are antiproliferative effect markers), survivin and Bcl2 levels (which are pro-apoptotic potential markers), and MMP-7 levels from the antimetastasis maker. In our study, it was noted that serum survivin and Bcl-2 values determined in the CCG rats were significantly higher than the values in the other groups. Similar to the results reported by Ahmed et al. (2), we noted that the serum CCSA-3 value, which is one of the colon-specific antigens determined in the rats in the

CCG, was higher than the values in the other groups. We interpreted the fact that serum survivin and Bcl-2 levels, which are inflammation and proapoptotic markers determined in FU-HG rats, were significantly lower than the value determined in CCG rats, as the positive effect of the combination of 5-FU and Turkish mad honey.

In the MNU-induced colorectal cancer models, Al-Hassan and Atawodi (4), found a significant difference between the groups in terms of neutrophil/lymphocyte ratio values, while Osowole et al. (31) found no significant difference in the hematological parameters between the groups. Hazilawati et al. (21), found a significant increase in the total leukocyte and lymphocyte levels determined in the groups in which MNU was applied, compared to the values in the control group, in the leukemia model they created by applying MNU in rats. Consistent with reports from various investigators (4, 21, 31). WBC, lymphocyte, eosinophil, and basophil values thought to be related to MNU administration were significantly lower in CCG rats than those determined in CG rats. The fact that WBC and lymphocyte values in FU-HG rats were significantly higher than the values determined in CCG rats was attributed to the combined effect of Turkish mad honey and 5-FU. In the necropsy of the rats in our study, the detection of hyperplastic changes in lymphoid follicles in the spleen tissue and the presence of free erythrocytes in some follicles were interpreted as the degenerative effect of MNU application, in accordance with the literature data (15, 21, 31).

Similar to the reports of researchers (27, 36, 38). pointing out that there are significant changes in LDH value in the colon cancer model in rats, in our study, it was determined that the serum LDH value determined in CG rats differed significantly ($P < 0.001$) from the values determined in CCG rats and HG rats. In our study, as a result of the evaluation of serum AST, ALT, total protein, albumin, urea, creatinine levels and histopathological examination results, we determined that there were no significant changes in liver and kidney functions in the process of cancer formation in rats using MNU or during the short-term treatment with Turkish wild honey and 5-FU.

Studies in which tissues were evaluated in colon cancer model studies created with MNU in rats were also carried out. Ahmed et al. (2), stated that they observed the therapeutic effect of punica peel extract at the tissue level in their histopathological evaluations of colon tissue. Huang et al. (22), reported that the efficacy of omega-3 PUFA in colon cancer tissue can be demonstrated by histopathological and protein-level evaluations. In our study, in the evaluation of nuclear atypia, mitotic figures, pleomorphism, polychromasia, vascularization, precancerous changes, and inflammatory infiltration, it was found that the increases in the values in the CCG rats were significantly different from those in the CG rats. In addition, we noted that nuclear atypia, mitotic figures, pleomorphism, polychromasia, vascularization, and precancerous changes were significantly decreased in the FU-HG rats, unlike the values in the CCG rats.

As a result, in the experimental colon cancer model created using MNU in rats, the use of Turkish mad honey and 5-FU separately and in combination reduced the development of anaplastic cells. It was observed that it decreased oxidative stress by reducing and suppressing anti-apoptotic activity, increased inflammatory activity in the intestine, and developed a response to anaplastic development. In addition, it was determined that there was no toxicity in the metabolism of Turkish mad honey or 5-FU due to the rare occurrence of liver and kidney damage in these groups. Therefore, it is thought that their combined use may be an alternative treatment in colon cancer. In addition, it is thought that the present study will guide the research on value-added and research and development products to be used in colon cancer.

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Conflicts of interest

The authors declare no conflict of interest.

Author Contributions

EK, MEA, AMA, BB, AKK and EG conceived and planned the experiments. EK, MEA, AMA, BB, AKK and EG carried out the experiments. EK, MEA, AMA, BB, AKK and EG planned and carried out the simulations. EK, MEA, AMA, BB, AKK and EG contributed to sample preparation. EK, MEA, AMA, BB, AKK and EG contributed to the interpretation of the results. EK, MEA, AMA, BB, AKK and EG took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

Permission to conduct the study was granted by the ethics committee decision numbered 2020-7-55 of Ankara University Animal Experiments Local Ethics Committee, Türkiye.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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