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Immunohistochemical Study of the Effect of Chamomile Extract on 5-Fluorouracil Induced Intestinal Mucositis in Albino Rats

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Abstract

Background and objectives: 5-Fluorouracil (5-FU) is a commonly used drug for the treatment of malignant cancers. Approximately 80% of patients undergoing 5-FU treatment suffer from gastrointestinal mucositis. The aim of this study was to investigate the effect of chamomile extract on the pathogenesis of 5-FU induced intestinal mucositis in Albino rat.

Materials and methods: In current study forty females Albino rats, weighing 220-280 g were used in the study. For the induction of mucositis, 60 mg/kg of 5-FU was administered intraperitoneally to each animal in the study group on day 0, and 40 mg/kg was administered on day 2. The control animals were intraperitoneally injected by normal saline in the same manner and dose like 5-FU on day 0 and 2. Then the rats in each group were randomly divided into two groups: Distilled water treated group and chamomile extract treated group (10 animals each).

A volume of distilled water equal to chamomile extract was given by intragastric gavage tube, while the other group was gavaged with chamomile extract at a dose of (100 mg/ kg) two times daily. The treatment with distilled water or the chamomile extract was initiated on day 5 and the experiment continues for twelve days. The body weight for each rat was measured and then the animals were sacrificed on day 8 and 12 (five animals each). In each experiment, one centimeter of proximal jejunum was removed for histopathological, intestinal morphometry, and immunohistochemical analysis using Ki-67 and Bcl-2 immunolabeling.

Results: Chamomile can protect the jejunum from fluorouracil-induced cytotoxicity and attenuate or decrease the associated injury. The chamomile in 5-FU/chamomile group causes significant increase in villi length, crypt depth, number of goblet cells, and Ki-67 and Bcl-2 immunexpression in comparison with 5-FU/water group at day 8. But longer duration of taking chamomile can cause cytotoxic and damaging effect to the jejunum.

Conclusion: Chamomile can protect the jejunum from fluorouracil-induced mucositis, it attenuate the associated injury if it taken for short duration, but the reverse was occurred if it taken for longer period.

Keywords: Mucositis; Jejunum; Chamomile; 5-FU

Introduction

Mucositis, also referred to as mucosal barrier injury, is one of the most debilitating side effects of chemotherapy treatment, characterized by inflammation, mucosal ulceration, and intestinal permeability which is probably due to villous atrophy [1]. Clinically, mucositis is associated with bacteremia, malnutrition, and the use of total parenteral nutrition. These complications all lead to longer hospitalizations and increasing health care costs. Moreover, mucositis is a frequent reason for reducing the dosages of chemotherapeutics or to postpone chemotherapy treatment, ultimately leading towards a higher mortality in cancer patients [2].

More recently, attention has been drawn towards the pathophysiology and clinical symptoms of intestinal mucositis [3], which is characterized by symptoms like nausea, bloating, vomiting, abdominal pain, and severe diarrhea [4,5].

According to the model introduced by Sonis, five phases are important in the pathophysiology of mucositis: The formation of reactive oxygen species leading to the activation of nuclear factor kappa B (NFκB) during the initiation phase, the induction of messenger molecules such as tumor necrosis factor alpha (TNFα), resulting in treatment-related tissue inflammation and apoptosis during the up regulation/message generation phase, the amplification of messenger molecules in the amplification/signaling phase, leading to more inflammation and apoptosis, discontinuity of the epithelial barrier resulting from apoptosis during the ulcerative phase, thereby promoting bacterial translocation, and a spontaneous healing phase, characterized by cell proliferation [1].

5-Fluorouracil (5-FU) is the most commonly used chemotherapy drug in the clinical oncologic practice. It is an antimetabolite drug and is considered one of the most commonly used chemotherapeutic agents; it is derived from a naturally occurring pyrimidine uracil in which a hydrogen atom at C-5 position is replaced by a fluorine atom [6]. It is widely used for the treatment of various cancers, including gastrointestinal cancer, breast cancer, and head and neck cancer. The common side effects of 5-FU include myelosuppression, dermatitis,

cardiac toxicity, diarrhea, and mucositis [7]. Mucositis of the intestine is characterized by increased crypt apoptosis and villus atrophy, leaving the mucosal tissue open to ulceration and infection [8].

Chamomile is a well-known medicinal plant species from the Asteraceae family often referred to as the "star among medicinal species." Its multi therapeutic, cosmetic, and nutritional values have been established through years of traditional and scientific use and research [9]. About one hundred twenty chemical constituents have been identified in chamomile, including terpenoids, flavonoids and coumarins [10]. Pharmacological action of it includes anti bacterial and antifungal action [11], antiviral [12], anti-inflammatory [13], antioxidants [14], anti cancer activity [15], and enhance wound healing [16].

Chamomile is used traditionally for numerous gastrointestinal conditions, including digestive disorders, "spasm" or colic, upset stomach, flatulence, ulcers, and gastrointestinal irritation [17]. Chamomile has immunmodulatory effect [18]. The aqueous extract of chamomile and chamomile oil exhibited significant anti-ulcer activity in ulcer induced models compared with omeprazole as standard drug [19]. Chamomile tea as a mouth wash can be used for treatment of methotrexate induced oral mucositis [20].

Currently, no successful intervention exists to treat intestinal mucositis completely. Any measure to decrease the frequency and or severity of stomatitis would be of obvious benefit, it may allow a greater opportunity for tumor response if higher doses of chemotherapy could be safely given. Thus, the aim of this study was to investigate the effect of chamomile alcoholic extract on the pathogenesis of 5-FU induced intestinal mucositis in Albino rat.

Materials and Methods

Rats and housing

In current study forty females Albino rats, weighing 220-280 g were supplied and cared in the Animal House of College of Medicine, Hawler Medical University, Erbil, Kurdistan Region of Iraq. The animals were kept under a standard laboratory conditions and maintained on a 12 hour light/dark cycle at 20 ± 5°C. They fed with a standard rat chow and allowed to drink water ad libitum. The research project was approved by the Research Ethics Committee at College of Dentistry, Hawler Medical University under protocol.

Induction of mucositis

For the induction of mucositis, 60 mg/kg of 5-FU (Kocak farma / Turky) was administered intraperitoneally to each animal in the study group on day 0, and 40 mg/kg was administered on day 2, following the protocol proposed by Sonis et al. [21] and modified by Leitao et al. [22].

Experimental design

The rats were randomly divided into two groups:

Control groups (Normal saline groups): Consist of distilled water treated group and chamomile extract treated group (10 animals each). In the distilled water treated group, a volume of distilled water equal to chamomile extract was given by intragastric gavage tube, while the chamomile extract-treated group was gavaged with chamomile extract (Matricaria recutita organic alcoholic extract-United States-Code HS3751002) at a dose of (100 mg/ kg) two times daily [23]. The animals were intraperitoneally injected by normal saline in the same manner and dose like 5-FU on day 0 and 2, and the treatment with distilled water or the chamomile extract was initiated on day 5 and the experiment continued for twelve days.

Study groups (5-FU groups): After induction of mucositis, the animals in this group were also divided in to two groups: the distilled water treated group and chamomile extract treated group (10 animals each) and treated in the same manner like the control group. The body weight for each rat was measured daily for all groups in the study.

Histopathological analysis

The animals were sacrificed by over dose of anesthesia on day 8 and 12 (five animals each) and the anterior abdominal wall was opened by a midline incision and the proximal jejunum was dissected. In each experiment, one centimeter was removed for histopathological analysis, fixed in 10% phosphate-buffered formalin, processed, and then embedded in paraffin, cut into 5-µm sections, stained with hematoxylin and eosin and trichrome stain. The mean number of goblet cells was counted at a magnification (x400) in 5 fields from section of each segment from each animal in all groups.

Intestinal morphometry

Villus height was measured from the baseline to the villus tip. Crypt depth was measured from the baseline to the submucosa [24]. Measurements of villus height and crypt depth determined from 40 villi and 40 crypts from four cross sections per tissue segment and a mean value was then obtained. The height were measured from hematoxylin and eosin slides on a light microscope (Olympus, Tokyo, Japan) equipped with a high-resolution digital camera (Microscope Eyepiece Camera UCMOS05100KPA/ Ver. 3.2) connected to a computer with an image captured program. All morphometric measurements were done blindly and were measured in well-orientated sections from digitized images that were evaluated at x10.

Immunohistochemical staining and analysis using Ki-67 and Bcl-2 immunolabelling

Cell proliferation was assessed by Ki-67 immunohistochemistry, while the anti apoptosis was assessed by Bcl-2 immunostaining and were performed using monoclonal Mouse Anti-Human Ki-67 Antigen, Clone MIB-1, Code No. M 7240 staining system, and a monoclonal Mouse Anti-Human BcL-2 Oncoprotien Clone 124 Code No 1587 ready to use N-series primary antibody, for use with Dako EnVisionTM , EnVisionTM double staining and LASABTM 2 systems. The staining procedure sections of the instructions included with each detection system were followed. Positive and negative controls were run simultaneously with biopsy specimen.

Positive cells expressing Ki-67 were identified by brown nuclei, while Bcl-2 was demonstrated brown cytoplasmic staining. To ensure the objectivity of the analysis, the evaluation was carried out by two independent observers. Five sections were randomly chosen for each animal. Approximately 1000 cells from cell population were counted by two observers at a magnification of 400x and the percentages of Ki-67 and Bcl-2 positive cells were calculated. All microscopic analyses were performed using a light microscope (Olympus, Tokyo, Japan).

The level of Ki-67 and Bcl-2 expression was evaluated according to the scoring system of Seleit et al. [25]. The application of this system

gives a score ranging from 0 to 3 for both degree of positivity: percentage of positively stained cells [(absent:<1%), (mild: 1-10%), (moderate: 10-50%), (strong:>50%)].

Statistical analysis

Statistical analysis was performed with one-way analysis of variance (ANOVA). P value<0.05 was considered statistically significant.

Result

Body weight

During the twelve days, the body weight increased in a stepwise fashion in the control rats of saline/water group. The body weight in the 5-FU/water group showed a tendency to decrease significantly ($p<0.05$) in comparison with other groups, but the reverse was occurred at day 12. The treatment with chamomile significantly ($p<0.05$) decreased the body weight at day 12 in the saline/chamomile and 5-FU/chamomile group in comparison with other groups as seen in Table 1. No significant difference ($p>0.05$) in the body weight was found between saline/water and saline/chamomile or 5-FU/chamomile groups at day 8, but it was significant ($p<0.05$) at day 12.

Histological findings

Saline/distilled water treated group: In the two studied periods (day 8 and day 12), microscopically the jejunum showed the normal appearance and structure, the mucosa of the jejunum, comprising simple columnar epithelium, forms finger-like projections, tall and cylindrical villi, which protrude into the lumen (Figure 1A1 and 1A2). The predominant cell in the epithelium is composed of columnar absorptive enterocyte cells with basally located nuclei which are evenly aligned. Interspersed between the enterocytes are the oval, mucins secreting goblet cells. The lamina propria contains mononuclear cells, smooth muscle fiber and blood vessels (Figure 1B1). The deep cavities, the crypts of Lieberkühn, are present between the villi (Figure 1B2).

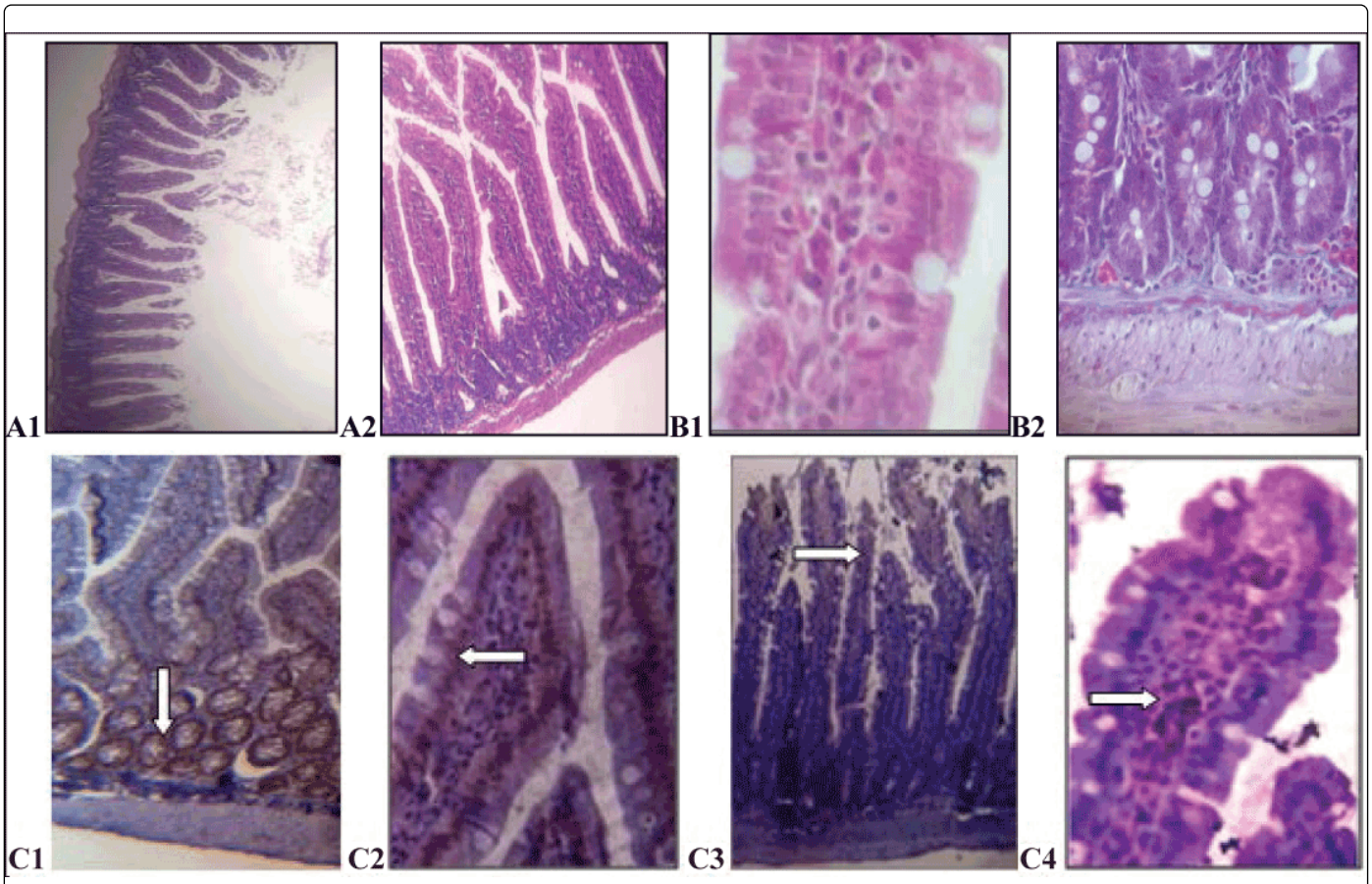

Figure 1: Saline/distilled water treated group: In the two studied periods, day 8 (A) and day 12 (B), the mucosa of the jejunum forms finger-like projections (A1, H&E x40, A2, H&E x100) comprising the epithelium and lamina propria. The predominant cell in the epithelium is composed of columnar cells with basally located nuclei which are evenly aligned. Interspersed between the enterocytes are the oval, mucins secreting goblet cells. The villi have a connective tissue core contain mononuclear cells, smooth muscle fiber and blood vessels (B1, Trichrome x400). Deep cavities, the crypts of Lieberkühn, are present between the villi (B2, Trichrome x400). Strong Ki-67 immuno reactivity in nuclei of cells of crypt, arrow (C1, immunohistochemistry x100) and villi, arrow (C2, immunohistochemistry x400). Moderate cytoplasmic reaction to Bcl-2 mostly seen in lamina propria, arrows (C3 immunohistochemistry x100, C4, immunohistochemistry x400).

Saline/chamomile extract treated group: At day 8, most of the villi and crypts are of normal length, and no congested blood vessels were seen in the lamina propria (Figure 2A1-2A3). Areas of missing epithelium with some cellular infiltration were seen in the core of some villi in the jejunum (Figure 2A4). But at day 12, the villi showed abnormal shape with loss of some villi (Figure 2B1). Necrosis in the tip of some villi, inflammatory cell infiltration in the lamina propria (Figure 2B2), and presence of congested blood vessels with decrease in the depth of crypt with muscular edema were also seen (Figure 2B3 and 2B4).

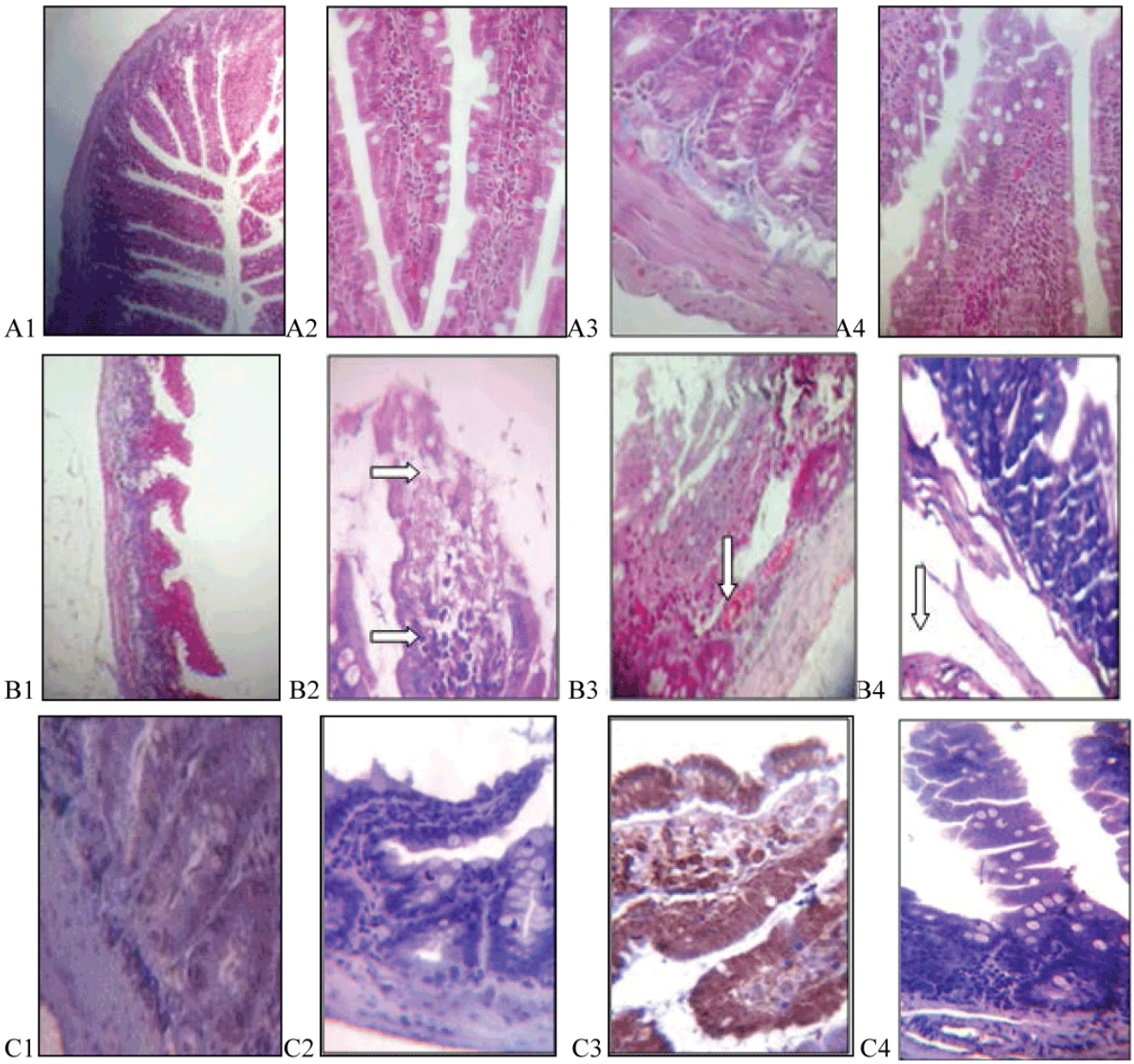

Figure 2: Saline/chamomile extract treated group: On day 8, the jejunum shows nearly normal shape of villi (A1, Trichrome x100), most of the villi seen cover by columnar epithelium, the crypt and the muscular layers appear normal (A2,A3 Trichrome x400), or sometimes the epithelium of villi are missing with cellular infiltration in the lamina propria (A4, Trichrome x400). On day 12, the villi appear with abnormal shape (B1, Trichrome x40), the tips of some villi shows necrosis (upper arrow) with inflammatory cell infiltration in the lamina propria, lower arrow (B2,H&E x400). Congested blood vessels in the lamina propria, arrow (B3, Trichrome x400) with muscular edema are also seen, arrow (B4,H&E x400). Mild and negative Ki-67 immuno reactivity in nuclei of cells at day eight and twelve respectively (C1, immunohistochemistry x400, C2, immunohistochemistry x400). Moderate and negative cytoplasmic reaction to Bcl-2 cells at day eight and twelve respectively (C3 immunohistochemistry x400, C4, immunohistochemistry x400).

5- FU / distilled water treated group: At day 8, 5-FU caused mucosal damage in the jejunum. 5-FU decreased the height of villi and cause fusion and blunting. Areas of complete loss of villi were also seen (Figure 3A1). Some of absorbing cells are lost in some area and the crypts are distorted (Figure 3A2). Moreover, 5-FU led to the intestinal inflammation, characterized by the infiltration of large number of inflammatory cells and dilation of congested blood vessels (Figure 3A3 and 3A4). At day 12, the villi of jejunum restore it shapes and height, they are lined by tall columnar cells, and most of the cells lining the crypts are more or less intact (Figure 3B1-3B3). The submucosa, muscularis mucosa, muscularis interna, and muscularis externa appear almost intact, few number of congested blood vessels were seen (Figure 3B4).

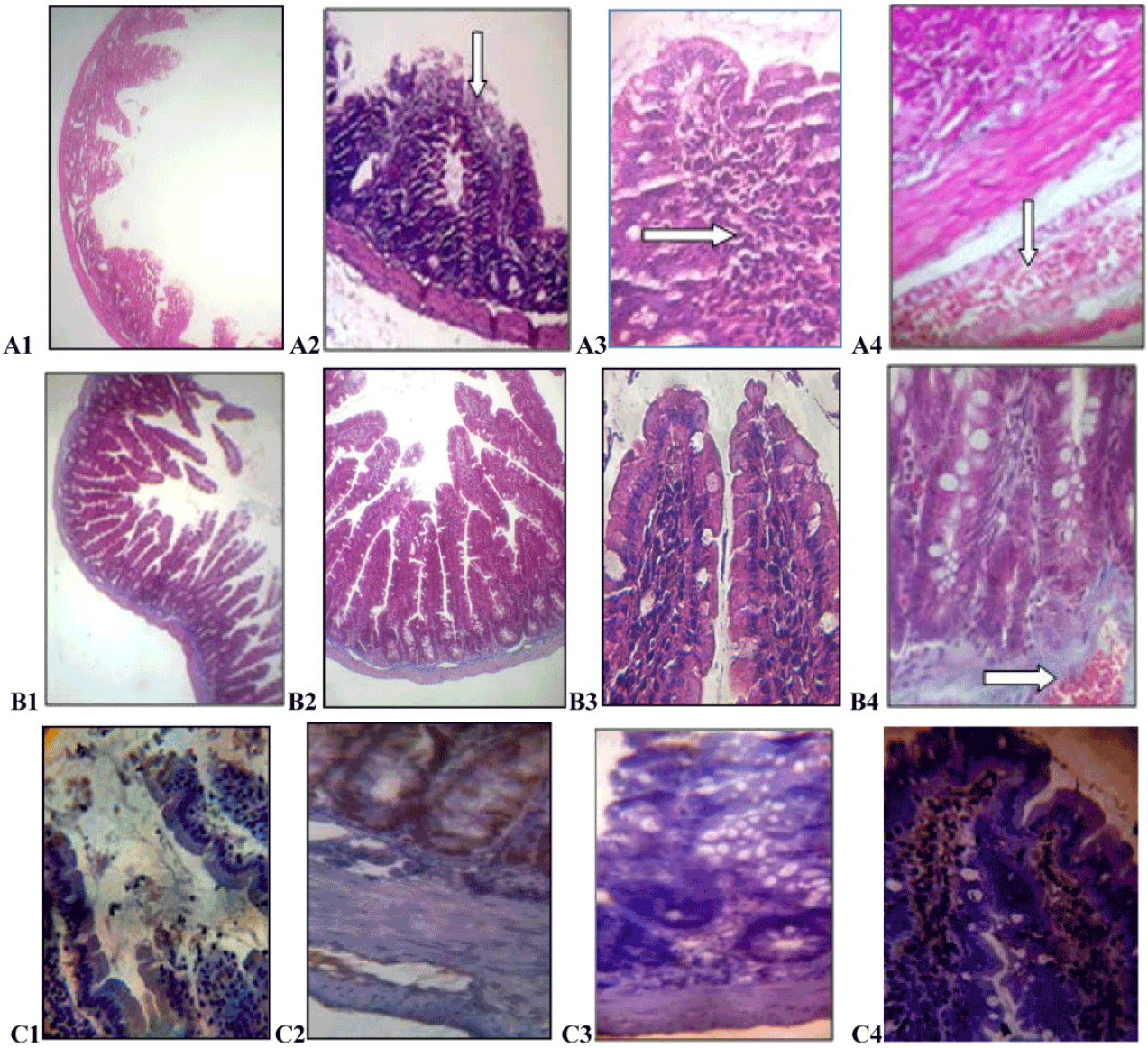

Figure 3: 5-FU/distilled water treated group: On day 8, 5-FU caused mucosal damage in the jejunum. It cause blunting of villi, they appear short and broad and sometimes missing (A1, H&E x40), some of the absorbing cells are lost in some area, arrow (A2, H&Ex100), and the goblet cells are markedly decreased in number with infiltration of inflammatory cells in the lamina propria, arrow (A3, H&E x400), muscular edema and congested blood vessels also can be seen, arrow (A4, Trichrome x400). On day 12, the villi of jejunum restore it shape (B1,Trichrome x40; B2,Trichrome x100), they are nearly intact and completely covered by tall columnar cells (B3, H&E x400). Nearly intact crypts with normal epithelial lining are also observed, but congested blood vessels also can be seen, arrow (B4, Trichrome x400). Negative and moderate Ki-67 immuno reactivity in nuclei of cells at day eight and twelve respectively (C1, immunohistochemistry x100, C2, immunohistochemistry x400). Negative and moderate cytoplasmic reaction to Bcl-2 cells at day eight and twelve respectively (C3 immunohistochemistry x400, C4, immunohistochemistry x400).

5-FU / chamomile extract treated group: At day 8, in comparison with 5-FU / water group at the same day, the appearance and structure of the small intestine were mostly preserved. The surface epithelium covering most of the villi was intact and continuous (Figure 4A1 and 4A2). Changes in the shape and areas of slight epithelial detachment were seen in some villi (Figure 4A3). Most of the cells lining the crypts are more or less intact. The submucosa, the muscularis mucosa, muscularis interna and the muscularis externa appear almost intact (Figure 4A4). At day 12, the villi appear with abnormal shape and are lost in some areas (Figure 4B1), the tip of villi may show necrosis (Figure 4B2), and the epithelium is completely separated from lamina propria in some areas or lost in other areas (Figure 4B3). The crypts are distorted with severe edema of the underlining muscular coat (Figure 4B4).

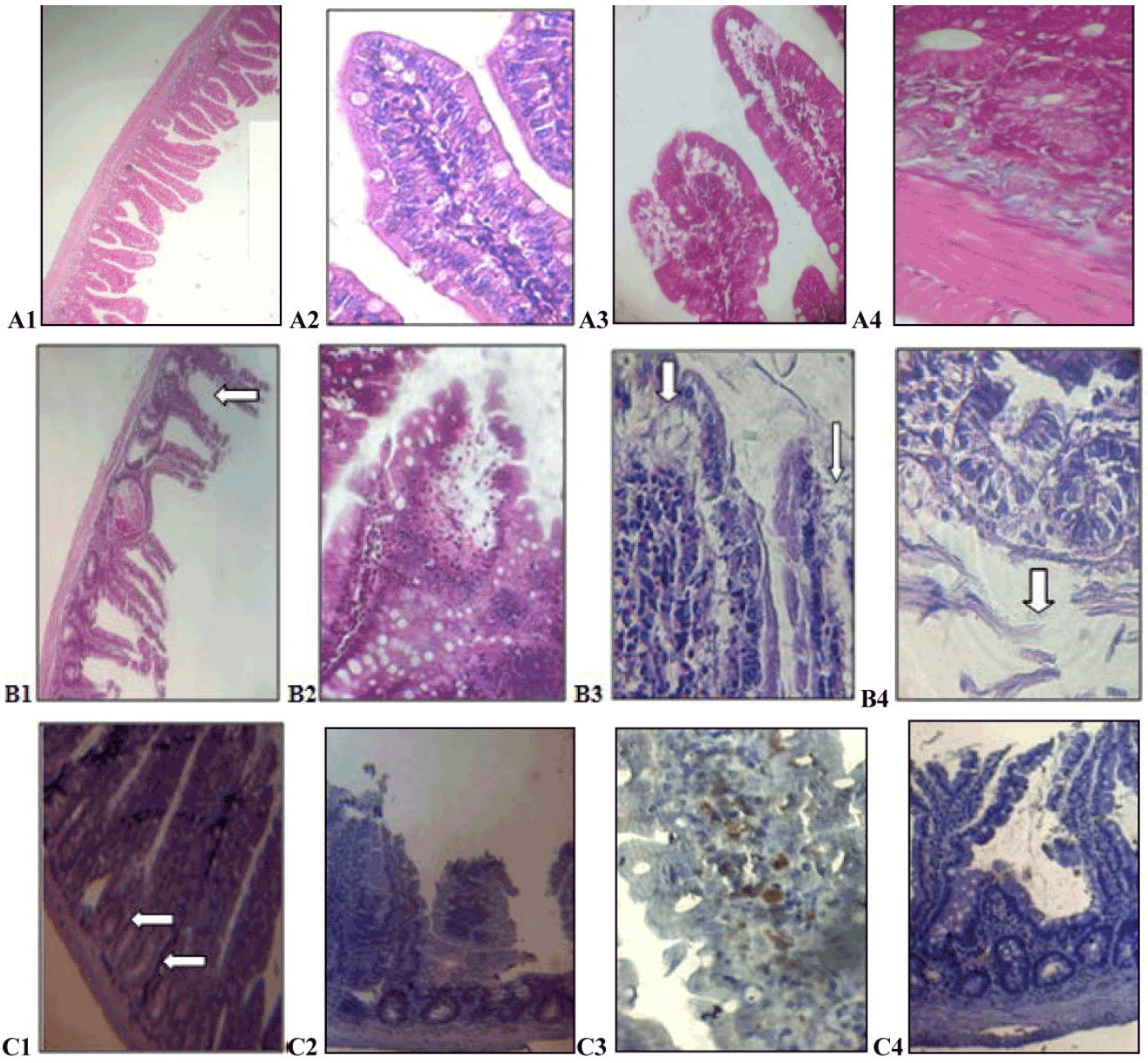

Figure- 4: 5-FU /chamomile extract treated group: On day 8, chamomile extract reduce villus height atrophy as well as crypt destruction (A1, H&E x10), most of the cells lining the villi are more or less intact (A2, H&E x400). Some villi showing changes in the shape with separation of the epithelium from lamina propria (A3, Trichrome x400). No muscular edema and congested blood vessels are seen (A4, Trichrome x400). On day 12, the villi are lost in some area, arrow (B1, Trichrome x 40), or show necrosis in other areas (B2, Trichrome x 400). The epithelium of villi may be separated from lamina propria (upper arrow) or lost in other areas, lower arrow (B3, H&E x400). The muscle layers shows severe edema, arrow (B4, H&E x400). Mild (arrows) and negative Ki-67 immuno reactivity in nuclei of cells at day eight and twelve respectively (C1, immunohistochemistry x100, C2, immunohistochemistry x100). Mild and negative cytoplasmic reaction to Bcl-2 cells at day eight and twelve respectively (C3 immunohistochemistry x400, C4, immunohistochemistry x100).

At day 8, significant decreases in villus height and crypt depth were observed in the jejunums of 5-FU/water rats compared with the other groups ($p<0.05$) in the same day. But at day 12, chamomile extract cause significant villus height and crypt depth decrease in the saline/chamomile and 5-FU/chamomile groups in comparison with the other groups ($p<0.05$) in the same day as seen in Table 2. No significant difference ($p>0.05$) in the villus height and crypt depth was found between saline/water and saline/chamomile or 5-FU/chamomile groups at day 8, but it was significant ($p<0.05$) at day 12.

At day 8, significant decreases in goblet cells number was observed in the jejunums of 5-FU/water rats compared with the other groups ($p<0.05$) in the same day. But at day 12, chamomile extract cause significant decrease in the number of goblet cells in the saline/chamomile and 5-FU/chamomile groups in comparison with the other groups ($p<0.05$) in the same day as seen in Table 3. No significant difference ($p>0.05$) in goblet cells number was found between saline/water and saline/chamomile or 5-FU-chamomile groups at day 8, but it was significant ($p<0.05$) at day 12.

Table 1: Means and standard deviations of body weight following water or chamomile extract treatment in female Albino rats post saline or 5-FU injection, in the studied periods of all groups in the study.

Weight (gm)		Saline/water (10 rats)	Saline/chamomile (10 rats)	5-FU/water (10 rats)	5-FU/chamomile (10 rats)
	Day 0	232.40 ± 9.67	239.20 ± 11.67	242.66 ± 12.81	249.62 ± 4.69
	Day8	241.40 ± 8.56	236.80 ± 6.49	203.40 ± 10.26	243.27 ± 7.08
	Day12	249.80 ± 5.11	208.60 ± 4.61	239.41 ± 5.59	211.69 ± 8.64

Table 2: Means and standard deviations of villus height and crypt depth of jejunum following water or chamomile extract treatment in female Albino rats post saline or 5-FU injection, in the studied periods of all groups in the study.

Mean & SD		Saline/water (10 rats)	Saline/chamomile (10 rats)	5-FU/water (10 rats)	5-FU/chamomile (10 rats)
Villus height (μm)	Day 8	337.40 ± 7.66	329.66 ± 6.16	213.80 ± 3.34	332.60 ± 4.33
	Day 12	338 ± 8.24	225.4 ± 6.22	328.2 ± 4.14	252.6 ± 5.41
Crypt depth(μm)	Day 8	73.6 ± 1.14	69.2 ± 2.16	37.6 ± 3.04	66.6 ± 0.54
	Day 12	75.8 ± 0.83	42.4 ± 2.70	72.4 ± 1.51	39.4 ± 1.51

Table 3: Means and standard deviations of goblet cells number in the jejunum following water or chamomile extract treatment in female Albino rats post saline or 5-FU injection, in the studied periods of all groups in the study.

Mean & SD		Saline/water (10 rats)	Saline/chamomile (10 rats)	5-FU/water (10 rats)	5-FU/chamomile (10 rats)
Number of goblet cells	Day8	116.9 ± 6.5	97.7 ± 2.9	23.6 ± 1.2	102 ± 6.4
	Day12	121 ± 1.4	36.2 ± 0.7	109 ± 2.7	33 ± 0.98

Table 4: Means and standard deviations of immunohistochemical results of the jejunum following water or chamomile extract treatment in female Albino rats post saline or 5-FU injection, in the studied periods of all groups in the study.

Mean & SD		Saline/water (10 rats)	Saline/chamomile (10 rats)	5-FU/water (10 rats)	5-FU/chamomile (10 rats)
Ki-67 antigen-positive cell rate	Day 8	59.00 ± 5.6	9.68 ± 0.31	0.02 ± 0.12	5.23 ± 0.71
	Day12	57.71 ± 3.23	0.20 ± 0.01	36.4 ± 2.14	0.31 ± 0.03
Bcl-2 antigen-positive cell rate	Day 8	41.23 ± 2.60	44.23 ± 4.04	0.41 ± 0.01	9.1 ± 0.67
	Day12	39.11 ± 3.99	0.12 ± 0.21	37.64 ± 3.66	0.22 ± 0.11

Immunohistochemical findings: Immunohistochemistry results of the jejunum of control and experimental groups are expressed in Table 4. At day 8, the number of cells staining positive for Ki-67 and Bcl-2 in the 5-FU/water group showed a tendency to decrease significantly ($p<0.05$) in comparison with the other groups. But at day 12, chamomile extract cause significant decrease in the number of Ki-67 and Bcl-2 positive cells in the saline/chamomile and 5-FU/chamomile groups in comparison with the other groups ($p<0.05$) in the same day (Table 4). Significant difference ($p<0.05$) in the number of cells staining positive for Ki-67 was found between saline/water and saline/chamomile or 5-FU/chamomile groups at day 8 and 12.

At day 8, no significant difference ($p>0.05$) in the Bcl-2 immunostaining was found between saline/water and saline/chamomile group, and a significant difference ($p<0.05$) was found between saline/water and 5-FU/chamomile group at the same day. At day 12, a significant difference was found between saline/water and saline/chamomile or 5-FU/chamomile groups only.

Discussion

Nowadays, researches are focusing on exploring the pharmacological profile of compounds from natural origin, where promising results aroused. The research activity is growing because of the increasing recognition of the importance of intestinal mucositis. The chemotherapeutic agent (5-FU) is a widely used antimetabolite drug which acts by blocking DNA synthesis, its mechanism of action targets not only cancer cells, but all rapidly dividing cells such as cells of the gastrointestinal tract [26]. The jejunum appeared to be the most affected intestinal segment. It has been reported that 5-FU preferentially damages the upper small intestine due to the higher cell turnover [27].

The study showed that, fluorouracil administration was accompanied by a significant weight reduction at day 8. A similar finding was previously reported in 5-FU treated rats and was attributed to altered intestinal absorptive capacity [28].

Microscopic signs of intestinal mucositis were observed in 5-FU / water group. Different signs of mucosal damage such as distortion, fusion, shortening and blunting of villi were observed, with loss of surface epithelium and marked exfoliation of the villi. This was associated with a significant decrease in the villus height and crypt depth. These changes are consistent with those reported by Soares et al. [28] and Stringer et al. [29]. Marked detachment of surface epithelium was observed and is suggested to be due to stromal edema [28].

A significant decrease in goblet cell number was observed in 5-FU/water group, and this may be a consequence of early stem cell death which has been reflected on the renewal of all cell lineages including goblet cells. The heavy cellular infiltration and dilatation of congestion of blood vessels seen in the mucosa and submucosa are similar to those reported in small intestinal irradiation mucositis [30]. It was stated that chemotherapy increases the release of proinflammatory cytokines which cause tissue damage and inflammatory response resulting in increased subepithelial vascularity [31].

The study showed that, fluorouracil administration was accompanied by a significant reduction in Ki-67 and Bcl-2 positive cells at day 8. It seems that inhibition of DNA synthesis, DNA damage and the production of reactive oxygen species by chemotherapy impair the metabolism in progenitor cells and cause inhibition of mitosis and increase of apoptosis [1]. Wright et al. [32] also found that 5-FU administration results in increased apoptosis and decreased cellularity in the small intestine.

Signs of initial recovery with significant increase in crypt depth and mitotic count were detected in 5-FU/water group at day 12. This recovery was accompanied by an increase in goblet cell number, indicating a complete cell renewal and migration of goblet cells to the villi. The elongation of the crypts seems to be a sign of crypt regeneration via hyper proliferation after the initial crypts damage. Carneiro-Filho et al. [33] observed similar findings by day 8 after 5-FU injection in mice. Duncan and Grant [34] stated that the structure and functionality of the villi and absorptive surfaces of human gut can return to normal around 1 week after onset of chemotherapy.

There is good evidence that chamomile extract exert their protective effect by many different mechanisms. In the 5-FU/chamomile group, the mean rat body weight was significantly higher than that of 5- FU/water group at day 8. This could be attributed to the improved intestinal mucositis and subsequent improvement of intestinal absorptive function. The current microscopic study demonstrated preserved structural integrity with some mucosal damage in jejunum of the 5-FU/chamomile group, but less than that of 5-FU/water group. Significant increases in the mean villus height and crypt depth were observed in 5-FU/chamomile group, compared to the corresponding 5-FU /water group at day 8, indicating less intestinal damage. Chamomile treatment with 5-FU administration led to significantly reversal of the shortening and fusion of villi and atrophy towards near normal. This suggests that the beneficial effect of chamomile treatment is specially exerted early during damage and the initial recovery phase.

The mean goblet cell number was significantly higher in 5-FU/chamomile group, compared to the corresponding 5-FU/water group at day 8. This increase may be explained by increase in antiapoptosis and stem cell proliferation. This change indicates another protective mechanism of chamomile against the 5-FU-induced damage through the preservation of the intestinal mucous barrier integrity.

Anti apoptosis and proliferation can be determined in the intestinal epithelium by immunohistochemical methods. The Ki-67 and Bcl-2 immunostaining was significantly lowest in the 5 FU/water group than the 5-FU/chamomile group at day 8. Thereby indicating that chamomile increased intestinal proliferation and anti apoptosis. It is probable that this protective effect of chamomile may prove useful in clinical practice to prevent or decrease intestinal injury resulting from 5-FU treatment.

The efficacy of chamomile as a mucosa protective agent has only evaluated in a few studies and their results are controversial [35,36]. The chamomile plant contains many different substances with antibacterial and antifungal properties, as chamazulene, alpha bisabolol, bisabol oxides, spiroethers, and flavonides. Considering the local microbial colonization of damaged mucosal surfaces which occurs in the ulcerative phase of mucositis, the intervention with chamomile and the effects of above components might be responsible for the reduction of mucositis in our animals. Flavonoids act as antioxidents, enhance the effects of vitamin C, and strengthen connective tissue around capillaries [36]. Bhaskaran et al. found that chamomile possesses antioxidant and cytoprotective properties [37] while Drummond et al. [38] found that chamomile possesses antiinflammatory activity by reducing IL-6 and TNF-α production.

Curra et al. [39] study the effect of topical chamomile (mouthwash) on immunohistochemical levels of IL-1β and TNF-α in 5-fluorouracil-induced oral mucositis in hamsters. They found the group treated with chamomile had lower scores for both pro-inflammatory cytokines.

At the same time, chamomile exerts harmful effect to jejunum especially at day 12, due to the severe microscopical changes seen at this day in comparison with the saline /water group. The harmful effect of chamomile may come from its constituents, like bisabolol, volatile oils, anthemic and tannic acid, and chamazulene which can cause gastrointestinal symptoms. Cavalieri et al. [40] found that α-bisabolol is a small oily sesquiterpene alcohol, it is a pro-apoptotic agent and enhance apoptosis. One study using cadaver skin demonstrated that Bisabolol can enhance the penetration of 5-

fluorouracil [41]. Kamatou and Viljoen [42] found that Bisabolol was nontoxic in acute oral studies in rats, dogs, and monkeys. Short term oral exposure using rats did produce inflammatory changes in several organs and decrease body weight.

Chamomile is included in the "Generally Regarded as Safe" (GRAS) list by the FDA [43], and according to Medicine Net. com, chamomile seems safe when taken by mouth for short periods of time. The long-term safety of chamomile is unknown.

Conclusion

Fluorouracil chemotherapy has a deleterious effect on the jejunum leading to marked morphometric and microscopic changes. Chamomile can protect the jejunum from fluorouracil-induced cytotoxicity, attenuate or decrease the associated injury if it taken for short period. Longer duration of taking chamomile can cause cytotoxic and damaging effect to the jejunum.

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