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Assessment the Effect of Ellagic Acid on 5-fluorouracil-induced Intestinal Mucositis and Diarrhea in Mice[#]

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Abstract

The intestinal mucositis is defined as the inflammation and ulceration in the -gastrointestinal tract wall and in some case in the -oral cavity that can be caused by treatment with antineoplastic drugs like 5-fluorouracil, irinotecan and others; since, the intestinal mucositis can in turn cause series of undesirable symptoms like severe diarrhea, abdominal pain, uncomfortable stomach and other symptoms. The current study was designed to evaluate the effects of ellagic acid against 5-fluorouracil-induced intestinal mucositis and diarrhea in mice. Induction of the intestinal mucositis was induced by intraperitoneal-injection of 50mg/kg 5-fluorouracil daily for 4 consecutive days and then, assessment of intestinal mucositis was performed by measuring the level of the antioxidant enzyme [superoxide dismutase (SOD)], and levels of some pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-α); and the lipid peroxidation product, the malondialdehyde (MDA) in tissue homogenate, in addition to the histopathological scoring; moreover, the potential action of ellagic acid was further supported by histopathological examination. Pretreatment of mice with 10mg/kg ellagic acid produced significant improvement in the level of antioxidant enzymes, pro inflammatory cytokines, lipid peroxidation product in the tissue homogenate, and the histopathological scoring and examination compared to such levels in the induction/5-fluorouracil group. Results suggested that ellagic acid is effective in protecting mice from intestinal mucositis induced by 5-fluorouracil.

Keywords: Ellagic acid, 5-Fluorouracil, pro-inflammatory cytokines, Intestinal mucositis

تقييم تاثير حمض الإيلاجيك على التهاب الغشاء المخاطي المعوي المستحث بواسطة خماسي الفلوريد اليوراسيل في الفئران[#]

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الخلاصه

يُعرَّف التهاب الغشاء المخاطي المعوي بأنه التهاب وتقرح في جدار الجهاز الهضمي وفي بعض الحالات في تجويف الفم الذي يمكن أن يحدث بسبب العلاج بمضادات الأورام مثل خماسي فلوريداليوراسيل واايرينوتيكان وغيرها؛ منذ ذلك الحين، يمكن أن يسبب التهاب الغشاء المخاطي المعوي بدوره سلسلة من الأعراض غير المرغوب فيها مثل الإسهال الشديد وآلام البطن وعدم الراحة في المعدة وأعراض أخرى. تم تصميم الدراسة الحالية لتقييم آثار حمض الإيلاجيك ضد التهاب الغشاء المخاطي المعوي الناجم عن خماسي فلوريداليوراسيل والإسهال في الفئران. تم إحداث التهاب الغشاء المخاطي المعوي عن طريق الحقن داخل الصفاق بمقدار ٥٠ ملغم/كغم من خماسي فلوريد اليوراسيل يوميًا لمدة ٤ أيام متتالية، ثم تقييم التهاب الغشاء المخاطي المعوي عن طريق قياس مستوى الإنزيم المضاد للأكسدة [الديسموتاز الفائق (SOD)] ، ومستويات بعض السيتوكينات المؤيدة للالتهابات كعامل نخر الورم -ألفا (TNF-α) ؛ ومنتج بيروكسيد الدهون المالون ثنائي الألديهايد (MDA) ، بالإضافة إلى تهديف الأنسجة المرضية؛ علاوة على ذلك ، تم دعم التأثير المحتمل لحمض الإيلاجيك عن طريق الفحص التشريحي المرضي. أظهرت النتائج الى ان المعالجة المسبقة للفئران بـ ١٠ملغم /كغم من حمض الإيلاجيك أدت إلى تحسن كبير في مستويات -الإنزيم المضاد للأكسدة، -السيتوكينات المؤيدة للالتهابات، -منتج بيروكسيد الدهون ، وتحسن ايضا في التسجيل والفحص التشريحي المرضي مقارنة بتلك المستويات في مجموعة الحث بخماسي فلوريد اليوراسيل. أشارت النتائج إلى أن حمض الإيلاجيك فعال في حماية الفئران من التهاب الغشاء المخاطي المعوي الناجم عن خماسي فلوريد اليوراسيل.

الكلمات المفتاحية : حمض االأيلاجيك، خماسي فلوريد اليوراسيل، التهاب الأمعاء المعوي، السايتوكينات الخاصة بالالتهابات

Introduction

Researchers reported that, between 50 and 80% of patients received chemotherapeutic drugs are thought to have experienced intestinal mucositis, which is followed by the clinical sign of ulceration, abdominal pain and diarrhea[(1)] . The 5-fluorouracil (5-FU) can suppress the synthesis of DNA *via* an inhibition in the production of thymidylate; where, five stages of the 5-FU can induce change in

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the small intestinal mucosa, **the first stage** including antineoplastic drug induced generation and up-regulation of messenger signals, **in the second stage,** the signal produce *via* inflammatory mediators like TNF-α, interleukin-6 (IL-6), and interleukin-1 beta (IL-1β), ulceration of mucosa consider **the third stage**, and then amplified mucosal damage, beginning of the healing process is the final stage[2]. This maybe led to direct damage of DNA and increase in the reactive oxygen species (ROS) and more of transcription factors productions; and the epithelium lining then initiate in lose the integrity as a result from tissue damage and cells death[3]

All symptoms of intestinal mucositis will appear after about 4-14 days from receiving the 5-FU; moreover, some factors can increase the risk of intestinal mucositis evolving, such as the specific antitumor drug, dose of drug, duration of treatment, and frequency; and other factors can also affect the severity of mucositis, such as tobacco smoke, age, gender, overall health before treatment, and genetic factor [4].

Ellagic acid is a form of tannic acid group and it is a polyphenol compound that is naturally-found in some fruit and medicinal plants; and it has antioxidant and anti-inflammatory properties; moreover, researchers proved that the EA protect the liver against valproic acid and carbon tetrachloride (CCl_4)[5].

Furthermore, EA have the ability to act on electrophysiological activity against traumatic brain damage, and it has a role in neurodegenerative disease like Parkinson's disease (PD)[6].

Additionally, researchers reported that the EA can prevent several types of cancers/breast, skin, and other types; furthermore, it can -increase the antioxidant enzymes and -reduce the pro-inflammatory cytokines through different pathway such its effect on decreasing nuclear factor-kappa B (NF-κB) activity, effect on release the cytochrome c through the potentiate the mitochondrial membrane; and through these methods, which are followed by ellagic acid lead to increase the Caspase-3 and increases expression in the tumor suppressor protein (p21) and then activation of apoptosis and cell cycle regulation[7] .

Even so, the count of crypt cells can be reduced while the number of the lymphocyte cells can be increased in a specific area of the mucous membrane after high dose of 5-FU[8].

The primary source of new chemical entities is the use of natural products, and plants are the main natural product source; since, EA is a phenolic compound sourced from ellagitannins (ET), a macromolecular family wherein hexahydroxydyphenic acid residues have been esterified with glucose or quinic acid. Punicalagin isoforms make up the majority of overall tannins derived from pomegranate[9]. Also, EA has mucoprotective in the intestinal mucositis by antioxidant and anti-inflammatory effect [10, 11].

Aims of the study

This study was designed to evaluate the effect of two-consecutive doses of ellagic acid on 5-fluorouracil-induced intestinal mucositis

Materials and Methods

Chemicals and drugs

High Altitude natural Ellagic Acid Powder (90%) Extract is extracted from Galla Chinese's plant, 5- Fluorouracil 500mg /10 ml ampoules manufacture in Celon Lab by: Shree Medical store Pvt Ltd, Delhi, Dimethyl sulfoxide (DMSO) solvent obtained from warehouse chemicals of College of Pharmacy/ University of Baghdad

Reagents

The ELISA Kite for cytokines analysis (superoxide dismutase (SOD) enzyme, malondialdehyde (MDA), and (TNF-α) were purchased from jianging, Nanjing, China

Experimental animals

From the Animal House of the College of Pharmacy/Baghdad University, 40 Albino male mice weighing (25-42) gram were brought in the temperature and humidity, light/dark cycle were kept at normal levels for the animals, free standard food and water were available to them. The animals were randomly-divided into four (4) groups each with ten (10) mice as follows:

Group I: (Negative control, vehicle)/Mice were given 0.1ml normal saline (NaCl) and dimethyl sulfoxide (DMSO) as solvent for EA in 10% ratio (1ml DMSO and 9ml NaCl) as; since orally-administered by gavage needle.

Group II: (Positive control)/Induction Group. Mice received normal saline (NS) orally by gavage needle for 10 days and from 7th day, the animals injected with 5-FU intraperitoneally (50mg/kg) for 4 days, and then at day 11, mice were euthanized by diethyl ether and then by cervical dislocation.

Group III: Mice orally-administered 10mg/kg EA by gavage needle once daily for ten days; then on day 7, mice IP injected with (50mg/kg) 5-FU for 4 consecutive days.

Groups IV: Mice orally-administered 5mg/kg EA by gavage needle once daily for 10 days; and on day 7, mice IP-injected with (50mg/kg) 5-FU for 4 consecutive days [12].

Ellagic acid (EA) dissolved in DMSO/normal saline in a ratio of 10%; where, it was dissolved in 1ml DMSO and complete the volume to 10ml by add 9ml normal saline[13].

Tissue Collection:
Mice were sacrificed by cervical subluxation while under anesthesia/diethyl ether at the end of the experiment. Then the duodenum was taken out and washed with phosphate buffer saline (PBS); and a portion of it was retained in 10% neutral buffered formalin for histopathologic observation and portion of it for homogenization[14].

Tissue homogenate preparation

At day 11, after killing the animals under anesthesia/by diethyl ether, the duodenum was removed, and washed with cooled-buffered saline solution (BSS), and then it was homogenized using a homogenizer and then centrifuged at 5000 rpm for 15 minutes at 4 0C, then the supernatant was obtained and separated using a micropipette and stored at 18 0C until the day of the analysis for the measurement of the enzyme's activity.

Assessment of the Antioxidant Superoxide dismutase (SOD) Enzyme in the Duodenal tissue homogenate

The concentration of the antioxidant enzyme (SOD) in the homogenized tissues of duodenum was then determined by ELISA analysis; where, this method includes the use of the competitive enzyme immunoassay technique/the sandwich enzyme-linked immunosorbent assay technology; since, 48-well plate is pre-coated with an antibody (Ab); and then the wells were incubated with standards, test samples, and biotin-conjugated reagents. and the enzyme horseradish peroxidase (HRP)-conjugate in a pre-coated plate; then the substrate was added to the well and allowed to sit for 15 minutes in a dark area before being stopped by the addition of a stop solution, which caused the solution to turn yellow; and as the color intensified, the concentration of SOD in the sample can be then determined by spectrophotometry at (450 nm) in a microplate reader[15].

Measurement the lipid peroxidation biomarker malondialdehyde (MDA)

The MDA contents in duodenal tissue homogenates were quantified using the MDA kit depending on the ELISA method; since, by comparing the optical density (OD) of the samples to the standard curve, the MDA content in duodenum tissue homogenate samples can be calculated; where, MDA levels were measured in Nano grams per milliliter (ng/ml)[16].

Determination of tumor necrosis factor-alpha (TNF-α)

The quantitative sandwich enzyme immunoassay technique is used in this assay; where, the TNF-α specific antibody was previously-already coated onto a miniature plate; then, the samples and standards were pipetted into the wells, and any TNF-α available is captured by the immobile antibody; and following the removal of any unbound substances, the wells were then treated with a biotin-conjugated antibody specific for TNF-α; then after washing, the wells were treated with avidin conjugated Horseradish Peroxidase (HRP); and after removing any unbound avidin-enzyme reagent, a substrate solution was added to the wells, and the color was grow in a proportion to the amount of TNF-α bound in the first step; and when the color expansion was halted, and the color intensity was measured[17].

Histological score examination

Portion of the duodenum was removed and washed with 0.9% NaCl then fixed in 10% formalin solution for about an hour, and then dehydrated in a highly-concentrated ethanol solution for about an hour, and then covered in paraffin wax also for about an hour. Then a part of duodenal tissue about (4µM) thickness was cut and fixed on the glass slide for (H and E) staining. Then, under a light microscope and by the utilization of a calibrated micro meter at various magnifications, histological analysis was used to measure villus heights, crypt presence, or any change in the mucosa layer effected by 5-FU and treatment, and the score was red for each sample that was depend on histopathological grading system as in Table (2) to determine the severity of intestinal mucositis (five samples for each group)[18,19].

Table 2. Histological-criteria-used-to-establish-the-score-of-intestinal-lesions [18]

Score	Description of finding
0	Tissue is normal without change
1	Shortening of villi and inflammatory cell infiltrations with edema in mucous layer and loss of crypt shape and normal muscular layer.
2	Moderate inflammation, edema in the muscular and sub muscular layer.
3	Crypt necrosis, blunting of the villus, severe inflammatory cell infiltration, edema in the muscle layers and high liquid in muscular and sub muscular layer.

Statistical analysis

The mean and standard error of the mean (SEM) were used to express the data. The statistical significance of the differences between groups was determined using SPSS statistics version 25's one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test. P-values less than 0.05 were considered statistically-significant.

Results

Effect of ellagic acid (EA) on the level of superoxide dismutase (SOD) enzyme in the duodenal tissue homogenate of mice

Table (3) and Figure (1) showed that, 5-FU (**Group II** mice) caused a significant reduction (P<0.05) in SOD levels in the mouse duodenal tissue homogenate compared to such tissue level in **Groups (I)** and **(III)** mice (115.894±1.18, 217.65±5.20, 193.14±9.30).

In group of mice that orally-administered 10mg/kg EA prior to IP injection of 5-FU (**Group III**), there was an increment in the duodenal tissue level of SOD enzyme compared to such tissue level group of mice (**Group IV**)/that orally-administered 5mg/kg EA; where, the SOD tissue levels were **(193.14±9.30, and 164.258±3.55)** respectively as shown in Table 3.

Furthermore, in **Group IV** mice/Orally-administered ellagic acid in a dose of 5mg/kg, there is a significant increase (P<0.05) in the SOD tissue level compared to such enzyme tissue level in **Group II** mice/IP injected with 5-FU; where, the levels are respectively, (164.258±3.55, 115.894±1.18); but such enzyme level in **Group II** mice is less than the corresponding enzyme level in the duodenal tissue of **Group III** mice/ Orally-administered 10mg/kg ellagic acid prior to 5-FU (Table 3, and Figure 1).

Additionally, by comparing the tissue level of SOD between **Group III**/Orally-administered 10mg/kg EA prior to IP injection of 5-FU and **Group IV/** orally-administered 5mg/kg EA mice, there is a significant increase (P<0.05) in the SOD tissue level in Group III compared to such tissue level in mice of **Group IV** as shown in Table (3) (193.1408±9.30, 164.258±3.55).

Table 3. Effect of Ellagic Acid (EA) on [superoxide dismutase (SOD), malondialdehyde (MDA), and tumour necrosis factor-alpha (TNF-α)

Treatment group N=4	type of treatment	SOD Mean ± SD	MDA Mean ± SD	TNF-α Mean ± SD
I	Normal saline + DMSO	217.6571±5.20*(c)	0.5999±0.069*(c)	56.28±11.84*(c)
II	Normal saline +5-FU	115.894±1.18(a)(b)(c)	1.8831±1.013(a)(b)(c)	166.84±73.02(a)(b)(c)
III	Ellagic acid (10mg/kg) +5-FU	193.1408±9.30*(c)	0.6318±0.052*(c)	60.59±13.90(c)
IV	Ellagic acid (5mg/kg) +5FU	164.258±3.55*(a)(b)	0.6621±0.08*(a)(b)	65.40±7.79*(a)(b)

*****Significant difference (P<0.05) comparable to Group II
[a] Significant difference (P<0.05) comparable to Group I
[b] significant difference (P<0.05) comparable to Group III
[c] significant difference (P<0.05) comparable to Group IV

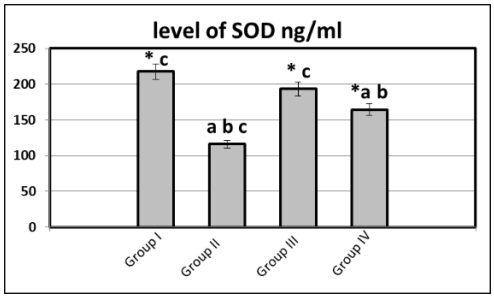


Figure 1. Effect of Ellagic Acid (EA) on superoxide dismutase (SOD) enzyme level in the duodenal mice tissue.

*Significant difference (P<0.05) comparable to **Group II.**[a] Significant difference (P<0.05) comparable to **Group I.** [b] Significant difference (P<0.05) comparable to **Group III**

[c] Significant difference (P<0.05) comparable to **Group IV**

Effect of Ellagic Acid (EA) on the Duodenal Tissue Malondialdehyde (MDA) level

Table 3 and Figure 2 showed that, IP injection of 5-FU (50mg/kg) for 4 days/ **Group II** caused a significant increase (P<0.05) in the duodenal tissue level of MDA compared to such level in the duodenal tissue of normal control/**Group I** mice.

Furthermore, in mice orally-administered EA (10mg/kg, and 5mg/kg each prior to 5-FU) **Group III**, and in **Group IV**, respectively, there were significant decrease (P<0.05) in the tissue level of MDA each compared to such tissue level in the model group [that IP injected with 50mg/kg 5-FU (**Group II**)]. Furthermore, by comparing of the level of MDA in the duodenal tissue between **Group III** mice orally-administered EA (10mg/kg) and **Group IV** (5mg/kg), there were significant decrease in such tissue level in the MDA in **Group III** mice compared to such level in **Group IV** mice (0.6318±0.052 vs 0.6621±0.08) as shown in Table (3) and Figure 2.

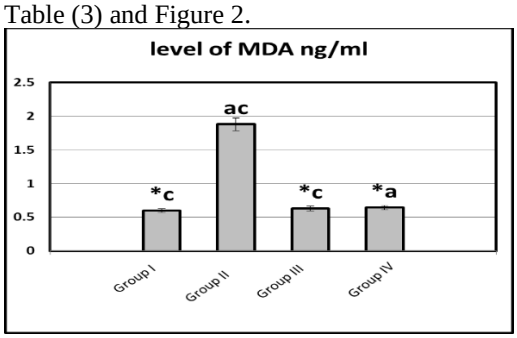


Figure 2. Effects of Ellagic acid on the malondialdehyde (MDA) level in the duodenum mice tissue.

*Significant difference (P<0.05) comparable to **Group II .**[a] Significant difference (P<0.05) comparable to **GroupI .**[b] Significant difference (P<0.05) comparable to **Group III .**[c] Significant difference (P<0.05) comparable to **Group IV**

Effect of ellagic acid on serum tumour necrosis factor-alpha (TNF-α level)

Table (3) and Figure (3) showed that, mice IP-injected with 5-FU (50mg/kg) for 4 consecutive days caused a significant increase (P<0.05) in the duodenum tissue TNF-α level compared to such level in the negative control **Group I** (166.84±73.02, 56.28±11.84).

Furthermore, Table (3) and Figure (3) showed that there was significant decrease (P<0.05) in the level of duodenal tissue TNF-α level in **Group III** mice/Orally-administered 10mg/kg of EA prior to 5-FU, compared to such tissue level in **Group II** mice/injected with 5-FU (60.59±13.90,166.84±73.02). Moreover, in **Group IV** mice/Orally-administered 5mg/kg EA prior to 5-FU, there were a reduction in the tissue level of TNF-α level compared to such tissue level in **Group II** (65.40±7.79, 166.84±73.02); additionally, by comparing the duodenal tissue TNF-α level between **Group IV** and **Group III** mice, there were significant increase in such tissue level in **Group IV** compared to that in **Group III** mice, (65.40±7.79, 60.59±13.90).

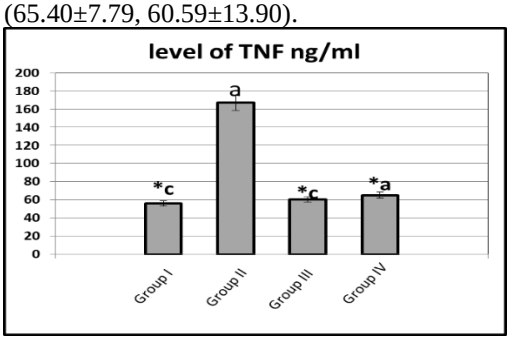


Figure 3. Effect of Ellagic acid (EA) on the tumour necrosis Factor-alpha (TNF-α) level

*Significant difference (P<0.05) comparable to **Group II .**[a] Significant difference (P<0.05) comparable to **GroupI .**[b] Significant difference (P<0.05) comparable to **Group III .**[c] Significant difference (P<0.05) comparable to **Group IV**

Effect of Ellagic acid (EA) on the Duodenal Tissue Histology

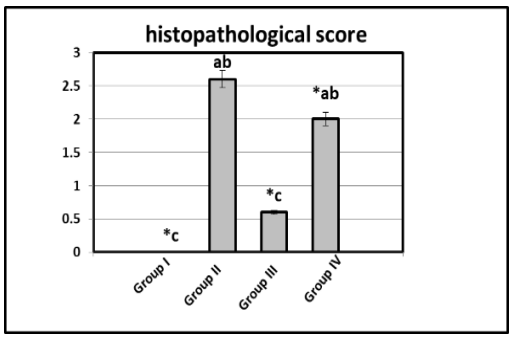
Table (4) and figures (4) and (5) showed that, in group of mice IP-injected with 5-FU/**Group II**, there was a significant increase (P<0.05) in the histopathological score comparable to those in normal control/**Group I** mice (2.6±0.54) and (0).

Furthermore, in **Group III** mice that received EA in a dose 10mg/kg prior to 5-FU, there is a significant decrease (P<0.05) in the histopathological score compared to such score in **Group II** mice/(IP injected with 5-FU); where, the scores are respectively, (0.6±0.54, and 2.6±0.54). Furthermore, by comparing in the histological scores between **Group III/Orally-administered 10mg/kg EA prior**

to 5-FU, and Group IV mice/Orally-administered 5mg/kg EA prior to 5-FU, there is a significant difference (P<0.05) in such scores between the two-mentioned groups of mice; where, levels are (2±0.70, and 0.6±0.54), respectively.

Table 4. Effect of Ellagic Acid (EA) on the scores of the duodenum tissue histology

Treatment group	Type of treatment	Score
I	Normal saline (NaCl) and Dimethyl sulfoxide (DMS) as solvent for ellagic acid	0
II	Normal saline and 5-FU	2.6±0.54
III	Ellagic acid 10mg/kg and 5-FU	0.6±0.54
IV	Ellagic acid 5mg/kg and 5-FU	2±0.70



Discussion

Cancer therapies like radiation, and chemotherapy, can cause irritation and disruptions of the lining of the GIT/mucous membranes, which is known as intestinal mucositis (IM); moreover, the cytotoxic treatment with chemotherapy can change both the structural and functional makeup of the GIT; since, common GI side effects from chemotherapy include heartburn, abdominal pain, diarrhea, bloating, and nausea[20].

The intestinal mucosal cells sustain direct harm from 5-FU and its active metabolite/fluorouridine triphosphate (FUTP), which results in the DNA damage and the production of ROS; additionally, researchers mentioned that 5-FU can activate a number of signaling pathways, such as the transcription factor/nuclear factor kappa-light-chain-enhancer of activated B cells (NF)-kB pathway, increase the expression of inflammatory mediators like prostaglandin E2 (PGE2) and cytokines associated with inflammation like TNF-α and IL-1β[21]

Additionally, a number of mechanisms were reported to be crucial in the pathogenesis of 5-FU-induced MI; since, it has been explained that, damage of the small intestine can be brought about by 5-FU; and these include oxidative- and nitrosative- stress, and increased levels of inflammatory factors that may promote apoptosis, which in turn could increase the inflammation, and destruction of intestinal tissue[22].

Researchers mentioned that the pathogenesis of IM can involve a combination of changes in villi height and crypt dimensions; and, the peroxidation, apoptotic inflammatory cells, the intestinal proliferation of cells, and gut microbiome composition can also worsen the condition; moreover, the pathogenesis of chemotherapeutic-induced mucositis was proposed by Sonis *et al* in 2004 as a five-phase process that included occurrence, expression and message creation, triggers and amplifying, ulceration, an inflammatory process, and then healing[23].

Furthermore, the variations occurred in the intestine and alter the structure of the such tissue (as seen in the shape of the villi, and the numbers of both the cell lumen and the inflammatory cell) were most noticeable during the IP injection of 5-FU. Moreover, Zhang *et al* [24] investigated the optimal dosage range of the maximum of five doses of 5-FU to cause mucositis in mouse models; thus, the present study also offered the following recommendations for the ideal mouse model in order to conduct any treatments or examinations on these animal studies; as it must closely-resemble human mucosal damage and have a reasonable survival rate; and thus the current study found that 5 doses of (50 mg/kg-100mg/kg) *via* intraperitoneal injection/day met the above criteria of the previous researchers; and that doses less than or greater than this concentration might cause insufficient tissue damage or a high rate of mortality, respectively.

Additionally, in the present study, EA orally-administered at a dose of 10mg/kg prior to 5-FU/**Group III**, give a good effect; where, it protects the intestine from the effect of 5-FU compared to such protective effects to the histopathological score of **Group II**/ mice IP injected with 5-FU; and in comparison, with those in **Group IV** mice.

Researchers mentioned that the damage to the small intestinal epithelial cells may make it more permeable, making it easier for microbes and harmful substances to pass through the compromised mucosal barrier and cause the development of IM; therefore, enhancing the bowel mucosal barrier's functionality could

represent an attractive target for lowering intestinal inflammation[25].

Moreover, researchers mentioned that EA have the ability to reduce the level of interleukin-6 (IL-6), interleukin-1beta (IL-1β), nitric oxide (NO), and TNF productions in the lipopolysaccharide (LPS)-induced RAW264.7 cells ; the macrophage-like cells, Abelson leukemia virus-transformed cell line derived from BALB/c mice which is a commonly used cells model of mouse macrophages for the study of cellular responses to microbes and their products; Thus, it protected the intestine from the chronic inflammation through its effect on pro-inflammatory cytokines[26].

In several experiments on animals, Singh *et al* (2009) , mentioned that EA protected against dextran sulfate sodium (DSS)-induced colitis in mice[27]; and, Ogawa et al. (2002) in DSS (dextran sulfate sodium)-induced UC (ulcerative colitis) in rats[28]; in addition to different animal and cellular models of IBD[29][30].

Furthermore, The mechanism of action of Ellagic acid is summarized as follows, EA present in the food as Ellagitannin (orally-administered) and can be metabolized by microbiota in the intestine and metabolized to active form (Urolithin A, B, C, D) and then absorbed by blood stream into different organ in the body like liver, kidney, intestine and spleen and act on different pathways such as mitogen-activated protein kinases (MAPKs) and NF-κB signaling pathways; and it can scavenge the free radical that cause the inflammation, then the EA act on caspases-3 and -9 to active the apoptosis process and then cell cycle regulation and improvement effect on pro inflammatory cytokines and antioxidant enzymes to reduce the inflammation and damage[31].

Conclusion

The current study found that EA significantly-reduced all aspects of 5-FU-induced IM that include some physical manifestation like the score of diarrheas, and it significantly-increased the antioxidant enzyme tissue (SOD), -decreased biochemical factors in the duodenal tissue like proinflammatory cytokines (TNF-α), and the lipid peroxidation (MDA) tissue level; also, ellagic acid significantly-improved histological scoring.

Conflicts of Interest

The authors declare there are no conflict of interest.

Funding

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Ethics Statements

The study received ethical approval from the Ethical Committee of the Faculty of Pharmacy, University of Baghdad, Iraq (approval #: 1221\12\2021).

Author Contribution

Conception and design of the study by Dr. Munaf H. Zalzala. Conducted animal experiment and manuscript writing by Dareen M. Al-hoshary

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