Original Article

Comparison of Effects of Different Doses of Resveratrol on Methotrexate Induced Toxic Effect on Jejunum in Adult Albino Rats

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ABSTRACT

Background: Methotrexate (MTx), a cytotoxic drug, causes severe damage of small intestine and alters its oxidative state. Resveratrol (RES) has a powerful anti-oxidant and anti-inflammatory activities. Aim of the study was to assess and compare the effects of different doses of Resveratrol on methotrexate induced jejunal toxicity. **Material and methods:** Forty Two adult male albino rats were divided into five groups; control group, RES treated group in which rats were subdivided into two subgroups and received low and high doses of resveratrol (RES) orally, MTx group in which rats were given methotrexate orally at a dose of 5 mg/kg daily for thirty days, MTx+ RES L group in which rats received low dose of RES with MTx dose , MTx+ RES H group rats were received RES in high dose combined with MTx dose. After 30 days of treatment, specimens of jejunum were obtained for histopathological, biochemical and immunohistochemical examination**.** **Results:** MTx induced epithelial and crypt cellular damage which was corrected by resveratrol and intestine nearly restored its normal structure, better results were gained with the high dose of resveratrol. Also, recovery of histopathological and biochemical findings was gained. **Conclusion:** this study concluded that Resveratrol could protect jejunum from damaging effect of methotrexate in a dose dependent manner.

Key words: Methotrexate, Resveratrol, oxidative stress, Jejunal toxicity, KI-67.

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I. INTRODUCTION

An analogue and antagonist of folic acid called methotrexate (4-amino-10-methylfolic acid, MTX) is frequently used in the treatment of a variety of malignant and non-malignant disorders. **(Chan and Cronstein, 2013).** Owing to its advantageous anti-inflammatory and immunomodulatory actions, MTX was initially created as an anticancer drug. It is now the first-line disease-modifying anti-rheumatic drug (DMARD) in the treatment of rheumatoid arthritis (RA), juvenile idiopathic arthritis, and psoriasis. It is also helpful in inflammatory bowel diseases, multiple sclerosis, vasculitis, systemic lupus erythematos **(Chan and Cronstein, 2010 & Chan and Cronstein, 2013].** MTX is widely used to treat a variety of autoimmune and inflammatory illnesses, however even when used at modest doses, it can still cause drug toxicity. According to reports, MTX can cause

mucositis in the intestines. Additionally, it has been demonstrated that the mucositis brought on by MTX in the rodent model is comparable to the damage to the human intestinal mucosa. **(Kolli et al., 2013).** It was found that MTX induced injury markedly affected jejunum more than other small intestinal parts (**Southcott et al., 2008).** So, this study chose rat jejunum to test the toxicity caused by MTx on intestine. The mechanism which causes the gastrointestinal toxicity of MTx is still incomprehensible. Yet, it has been found that inflammatory reaction and leucocytic infiltration were the main but not definitive cause of MTX induced intestinal toxicity **(Zhou et al., 2018).** Moreover, increased level of oxidation contents and decrease level of antioxidants were reported accompanying MTX administration **(Arslan et al., 2015; Kuyrukluyıldız et al., 2016).**

Resveratrol (trans-3,4',5-trihidroksi-stilben) (RES) is a polyphenol phytoalexin. It is found in various different plants, and particularly in grape, peanut and mulberry **(Harikumar & Aggarwal,2008) .** Research on the effects of RES has increased in recent years. Studies have emphasized its antioxidant, anti-inflammatory, antiplatelet, anti-atherogenic and cancer inhibiting effects **(Koushki et al., 2018).** RES scavenges reactive oxygen species (ROS), inhibits damage to DNA and lipid peroxidation in the cell membrane **(Heo et al.,2018).** RES proved to reverse oxidative stress in many tissues. **(Tomé-Carneiro et al., 2013& Yulug et al., 2013& Wang et al.,2020).** RES decreases oxidative stress induced ischemia reperfusion injury in the heart, kidney, brain, ilium, and retina **(Tomé-Carneiro et al., 2013; Yulug et al., 2013& Chronopoulos et al., 2023).**
Considering the role of oxidative stress in MTX associated intestinal damage and owing to powerful antioxidant and anti-inflammatory properties of resveratrol (RES) **(Gambini, et al., 2015),** so this study aimed to evaluate the role of RES as an antioxidant and protective agent in MTx chemotherapeutic regimens and compare the use of its different doses against methotrexate induced Jejunal toxicity.

II.MATERIAL AND METHODS

Animals:
Forty-two male albino rats who were 8 weeks old and weighed 200 ± 20 g were utilized in this experiment. They were properly housed at controlled accommodated circumstances; temperature, light/dark cycles, diet and water for 2 weeks prior to conducting the experiment. The procedures were examined and authorized by the Benha Faculty of Medicine's research ethical committee for animal care and use. (RC.19.12.2022).

Drugs:
Methotrexate drug (MTx) was obtained from Sigma (Sigma Chemical Co., USA), and the dosage was 5 mg/kg/day for 30 days to ensure induction of methotrexate intestinal toxicity according to (**Arslan et al., 2015**), it was dissolved in Dimethyl sulfoxide (DMSO). Resveratrol was obtained from Sigma (Resveratrol, R5010–500 mg, Sigma-Aldrich,St.Louis, MO, USA), dissolved in DMSO.

Experimental design
Forty-two rats were randomly divided into five groups (the control group and group II each was 12 rats, while other groups were 6 rats each) as follow:

- Group I (control group): 12 rats subdivided into 2 subgroups, each one containing 6 rats:
  Subgroup Ia: rats received nothing other than food and water, for 30 days.

Subgroup Ib: orally administered DMSO by gavage for 30 days, as the solvent used for MTx and Resveratrol groups.

Group II (RES treated group): 12 rats subdivided into 2 subgroups, each one containing 6 rats:

Subgroup IIa (RES L - treated group): rats were orally administered RES, at a dosage of (25mg /kg /day) dissolved in DMSO daily via gastric gavage for 30 days.

Subgroup IIb (RES H- treated group): orally administered RES, at a dosage of (50mg /kg /day) dissolved in DMSO daily via gastric gavage for 30 days (**Castro et al., 2021).**)

- Group III (MTx- treated group): MTx was given at a dose of (5 mg/kg/day) dissolved in DMSO by oral gavage daily for 30 days (**Arslan et al.,2015).**
- Group IV (MTx + RES L treated group): orally administered RES in a low dose (25mg /kg /day) dissolved in DMSO daily via gastric gavage **(Arslan et al.,2015; Castro et al., 2021).** Then, rats received MTx after an hour of RES administration and in the same way and dose as in group III.
- Group V (MTx + RES H treated group): received the same as in group IV but with a high dose of RES (50 mg/ kg / day).

At the end of the experiment the rats were sacrificed under diethyl ether. Half the jejunal tissue was fixed in 10 % formalin for histopathological evaluation and immunohistochemical examination, while the other half was placed in an Eppendorf tube and stored at –80 °C for biochemical investigation.

Biochemical sampling:

Estimation of Malondialdehyde (MDA): Measurement of MDA was performed according to **Uchiyama and Mihara, (1978)** procedures and expressed as nanomoles /gram tissue.

Estimation of Superoxide dismutase (SOD) activity: A segment of jejunum from each rat was homogenized in ice cold Tris HCL buffer. According to the nitroblue tetrazolium reduction inhibition rate, the activity of SOD could be determined (**Sun et al.,1988).** Degree of fifty % inhibition was agreed to count SOD activity as one unit. The expression was given as U/g tissue protein**.**

Histopathological preparation

The abdomen of each scarified rat was opened through a midline incision and jejunum (middle part of small intestine) of each rat was gently removed. Each jejunum was divided into many pieces, each of which will be used in the next different procedures. Segment's lumen was washed and cleaned with cold saline solution.

For light microscopic examination, segments were fixed in 10% buffered formalin solution for 24 hours. Successive sections of 5 µicron thickness were prepared from the paraffin blocks after the routine tissue monitoring procedures of dehydration and embedding. Then, sections were stained with hematoxylin & eosin stain (H &E); for general histologic picture, Alcian Blue stain for Goblet cells detection and immunohistochemistry evaluation; **(Datta et al.,2013; El-Sheikh et al.,2018).** Sections were finally evaluated under light microscopy (Olympus BX 52, Tokyo, Japan).

For Alcian blue stain, after being deparaffinized and put in distilled water, Sections were submerged in a 3% glacial acetic acid solution for 3 minutes. After that, they were submerged for 30 minutes in Alcian Blue stain (1% alcian blue in 3% glacial acetic acid; pH 2.5). Nuclear Fast Red (0.1%; Sigma, USA) was used then for 5 min as a counter stain. After, sections were soaked in water, dehydrated, and mounted. Finally examined histologically (**Sugiyama et al.,2011).**

Immunohistochemistry

Ki-67 antigen mouse monoclonal antibody, from Dakocytomation, Inc. Biotinylated Link Universal, North America, USA, was the primary antibody utilised for immunohistochemical investigation. The streptavidin-biotin-peroxidase technique was used to carry out the Ki-67 immunohistochemistry examination. All sections underwent dewaxing, rehydration, rinsing with Tris-buffered saline (TBS; pH 7.6), treatment with 3% hydrogen peroxide, and a final TBS rinse. To extract antigen, tissue pieces were placed in citrate buffer (pH 6.0) and autoclaved for 15 minutes. Without lifting the lid, the samples were held for around 45 minutes to come to room temperature. After then, primary antibodies were "dropped" and left in a wet, enclosed setting for 1.5–2 hours. The slides were counterstained with Mayer's hematoxylin following a second wash with TBS. (**Brandt et al.,2000)** .

Morphometric Study: Semi-quantitative analysis using image analysis was performed by counting the number of goblet cells per field under a light microscope. The mean number of such cells in 100 randomly selected fields in each Alcian blue stained jejunal tissue was expressed as the number of goblet cells. Also, the area % of KI67 positive immunoreactivity analyzed in 10 non-overlapping fields of each animal of Ki 67 stained jejunal sections **(Fernández-Gil et al.,2017)**.

Statistical analysis:

Version 22 (IBM Corp., Armonk, NY, USA) was used to record and analyze all of the experiment's data. To compare variations in the groups of morphometric results, one-way analysis of variance (ANOVA) with the Post Hoc Tukey test was performed. The mean (M) value and standard deviation (SD) of the data were used in each test, and differences were deemed significant at $P \leq 0.05$.

III. RESULTS

Biochemical results:

Biochemical data between Subgroups Ia & Ib were statistically analyzed and the difference of the results were found to be statistically insignificant, so Subgroup Ia results were applied to them.

Biochemical data between Subgroups IIa & IIb were statistically analyzed and the difference of the results were found to be statistically insignificant, so Subgroup IIb (RES H- treated group) results were applied to them.

MDA tissue level was highly significantly raised in MTx- treated group compared to control and RES- treated groups, MDA level was significantly diminished in MTx + RES L treated group as compared to MTx- treated group, but it was highly significantly decreased in MTx + RES H treated group as compared to MTx- treated group. MDA level in MTx + RES L treated group was significantly increased when compared to control and RES-treated groups, but insignificantly increased in MTx + RES H treated group in comparison to control and RES-treated groups.

SOD activity was highly significantly decreased in MTx- treated group compared to control and RES- treated groups. SOD activity was significantly elevated in MTx + RES L treated group as compared to MTx- treated group, but it was highly significantly increased in MTx + RES H treated group as compared to MTx-treated group. SOD level in MTx + RES L treated group was significantly decreased when compared to control and RES-treated groups, but insignificantly diminished in MTx + RES Htreated group in comparison to control and RES-treated groups (Table 1, Histogram 1).

Histopathological examination:

The jejunal H&E-stained sections of control groups (Ia) revealed healthy tongue-like villi

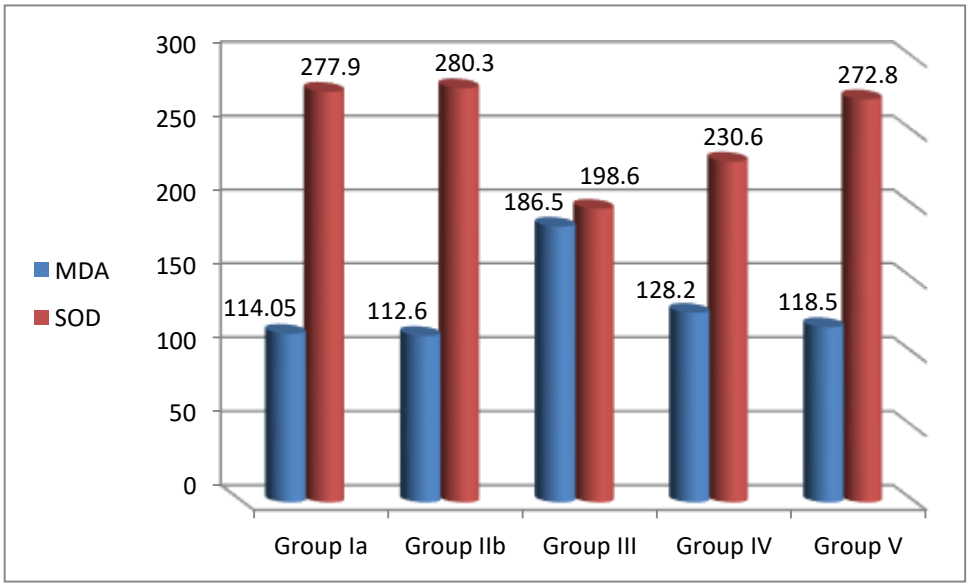
in the form of connective tissue core (lamina propria) covered by columnar enterocytes with basal cubical nuclei and mature goblet cells. Abundant crypts were present at the base of the villi, lined by absorptive and mature goblet cells and contained Paneth cells at their bottom **(Figure 1a).**

H&E stained jejunal sections of subgroups IIa & IIb showed no difference in the standard histological structure, so subgroup IIb (RES H-treated group) results were applied to them, it showed no pathological finding and nearly similar to control group **(Figure 1b)**.

Table (1): Statistical analysis of Malondialdehyde &Superoxide dismutase levels in the different studied groups

Groups / parameters	Control Subgroup Ia	Subgroup IIb (RES H-treated) group	Group III (MTx)-treated group	Group IV (MTx+RES L-treated) group	Group V (MTx + RES H-treated) group
MDA (nmol/ g protein)	114.05 ± 1.5$^{c \& d}$	112.6 ± 0.6 $^{c \& d}$	186.5 ± 4 $^{a,b, d \& e}$	128.2 ± 10.2$^{a.b \&c}$	118.5 ± 2.3 ^{c}
SOD (U / g protein)	277.9 ± 1.7 $^{c \& d}$	280.3 ± 0.8 $^{c \& d}$	198.6 ± 0.9 $^{a, b, d \& e}$	230.6 ± 9.4 $^{a.b \&c}$	272.8 ± 1.1 ^{c}

Number of samples = 6 in each group, Data expressed as mean ± SD, *: significance ≤ 0.05; One way ANOVA method followed by post-hoc Tukey's test; a: Significance vs Control, b: Significance vs RES H, c: Significance vs MTx, d: Significance vs MTx + RES L, e: Significance vs MTx + RES H.



Histogram (1): showing mean tissue levels of MDA & SOD in the studied groups

On the other side, MTx treated group sections showed damage of standard configuration of jejunum. Villi were degenerating with necrotic enterocytes and inflammatory cell infiltrate scattered through and burst out of ruptured villi. A lot of villi were sloughed and lamina propria was detached from covering epithelium. There is fusion of villi. Most crypts were degenerated with cellular debris and necrotic cells. Some crypts were tall with lined degenerated cells, other crypts were hypoplastic. Congested blood vessels were also seen. Hypertrophied musculosa was demarcated in this group. In addition, there was hyperplasia of covering epithelial cells and there were pyknotic nuclei **(Figures 1c & 1d)**.

In group IV (MTx + RES L treated) group, most of the villi attained their normal shape and covering epithelium. While some villi showed discontinuity of covering enterocytes with slouphed cellular debris into the lumen, there were some inflammatory cellular infiltrate at the base of the villi with few degenerated crypts **(Figure 1e)**.Whereas in group V (MTx + RES H treated) group, villous appearance showed nearly normal configuration, few slouphged villi were also monitored. Jejunal crypts showed normal appearance **(Figure 1f).**

Alcian Blue Staining Results:

Alcian Blue stained jejunal sections of control and RES H-treated groups revealed scattered goblet cells all over villi and crypts, cells which stained blue and indicating acid mucin produced by these cells **(Figures 2a&b)**. While MTx –treated group sections showed marked diminution of goblet cells in villi **(Figure 2c)**. Goblet cells were slightly increased in MTx + RES L treated group relative to that of MTx group **(Figure 2d)** but MTx + RES H treated group revealed scattered goblet cells all over villi and crypts **(Figure 2e).**

The mean number of goblet cells In MTx treated group stained by alcian blue showed a significant diminution in comparison with control and RES H – treated groups ($P \leq 0.05$). In MTx + RES L treated group; the mean number of goblet cells significantly improved when compared with MTx treated group ($P \leq 0.05$). The mean number of goblet cells was significantly elevated in MTx + RES H treated group when compared with MTx treated group ($P \leq 0.05$), but non-significantly reduced as compared to control and RES- treated groups ($p > 0.05$) (Table.2).

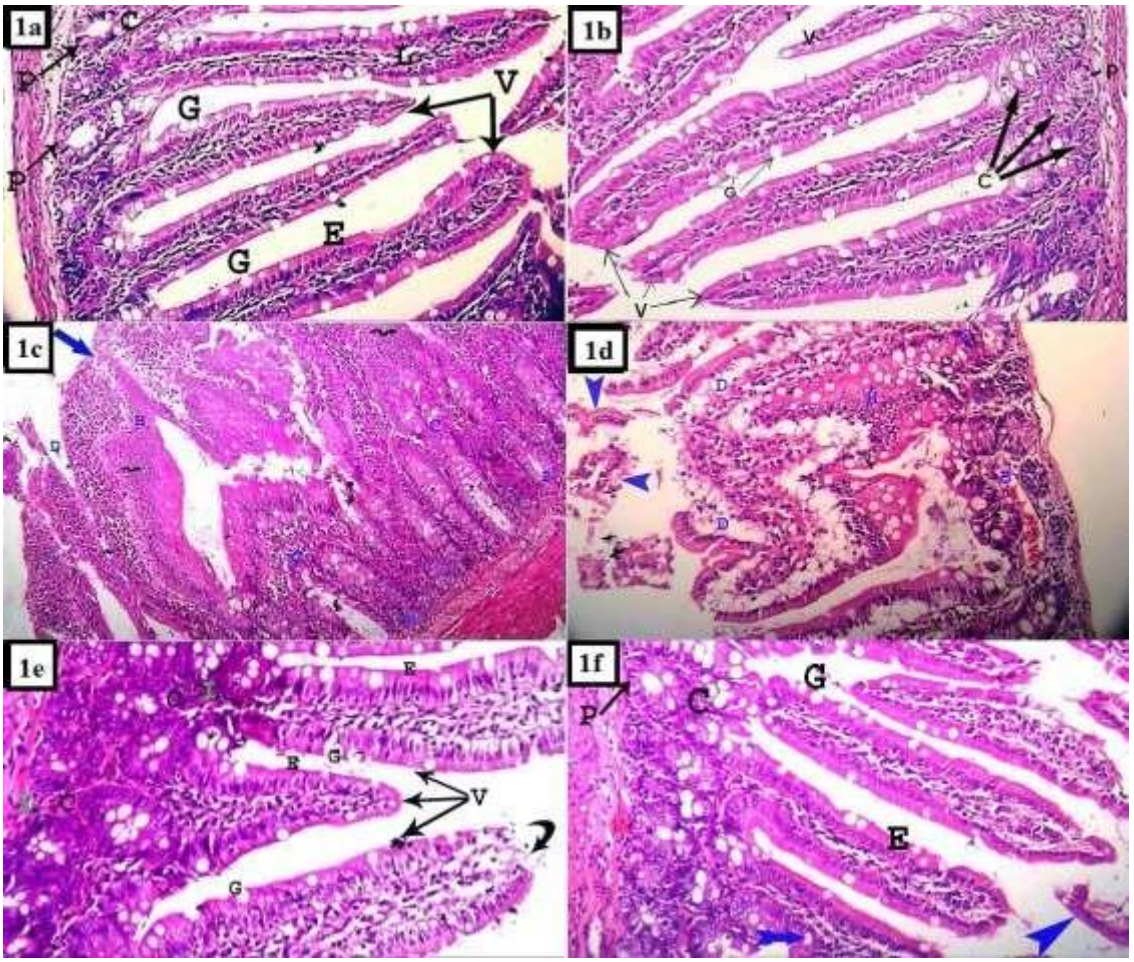


Figure. (1): photomicrographs of cross section of adult rat jejunum of: **(1a)** Group I (control group) showing tongue like villi (V) with covering enterocytes (E) and mature goblet cells (G), C.T core (lamina propria) (L), crypts of Lieberkuhn (C) at the base of villi with Paneth cells (P) at the bottom of the crypts**. (1b)** group II (RES H- treated group) showing tongue like villi (V) with covering enterocytes and mature goblet cells (G), crypts of Lieberkuhn (C) at the base of villi with Paneth cells (P) at the bottom of the crypts**. (1c)** group III (MTx group) showing fused villi (blue arrow), hyperplasia of cells (H) with some pyknotic nuclei (zigzag arrow), detachment of covering epithelium from underlying lamina propria (D), tall crypts (C) with degenerated cells, Paneth cells (P) that appeared ballooned with increased eosinophilic granules. **(1d)** group III (MTx group) showing hyperplasia of cells (H), detachment of covering epithelium from underlying lamina propria (D), hypoplastic crypts (C) with degenerated lining cells, necrotic cellular debris and inflammatory cells projected into the lumen (arrowhead) and congested bl. vessels (B). **(1e)** group IV (MTx+RES L) showing tongue like villi (V), covered by enterocytes(E) and mature goblet cells (G), some villi show erosion of covering epithelial cells (curved arrow), some crypts filled with necrotic cells (C) and there is some inflammatory cellular infiltrate (I) at the base of the villus and in between the crypts. **(1f)** group V (MTx+RES H) showing tongue like villi, covered by enterocytes(E) and mature goblet cells (G), but some covering epithelium shows interruption (pointed blue arrow), few cellular debris within the lumen (arrowhead). Crypts (C) lined by absorptive cells and mature goblet cells with Paneth cells (P) at their base. **(H&E X200)**

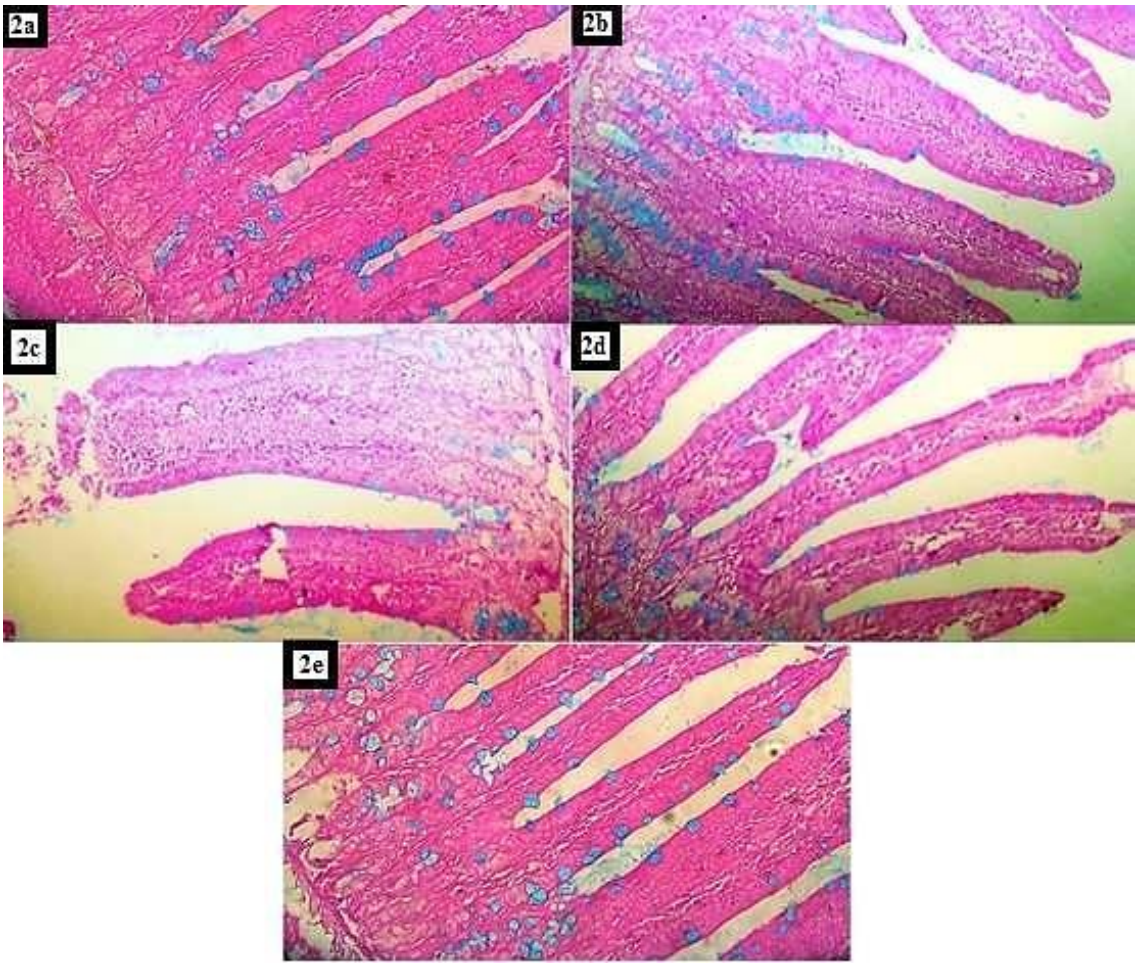


Figure. (2): photomicrographs of cross section of adult rat jejunum of: **(2a)** Control Group, and **(2b)** group II (RES H- treated group): demonstrating mature goblet cells mucin dispersed across villi and crypts**. (2c)** group III (MTx group): displaying obvious diminution of goblet cells among villi. **(2d)** group IV (MTx+RES L) showing increased number of goblet cells in the crypts and villi. **(2e)** group V (MTx+RES H) presenting scattered goblet cells across villi and crypts. **(Alcian blue X 200).**

Table (2): Statistical analysis of mean goblet number in the studied groups

Groups / **parameter**	**Control Subgroup Ia**	**Subgroup IIb (RES H)-treated group**	**Group III (MTx)-treated group**	**Group IV (MTx+RES L)-treated group**	**Group V (MTx+RES H)-treated group**
Goblet number	267 ± 2.5 $^{c \& d}$	269 ± 2.08 $^{c \& d}$	108 ±1 $^{a,b,d \& e}$	248 ± 1 $^{a,b \& c}$	263 ± 3.06 ^{c}

Number of samples = 6 in each group

Data expressed as mean±SD, *: significance ≤ 0.05

One way ANOVA method followed by post-hoc Tukey's test

a: Significance vs Control, b: Significance vs RES H, c: Significance vs MTx, d: Significance vs MTx + RES L, e: Significance vs MTx + RES H.

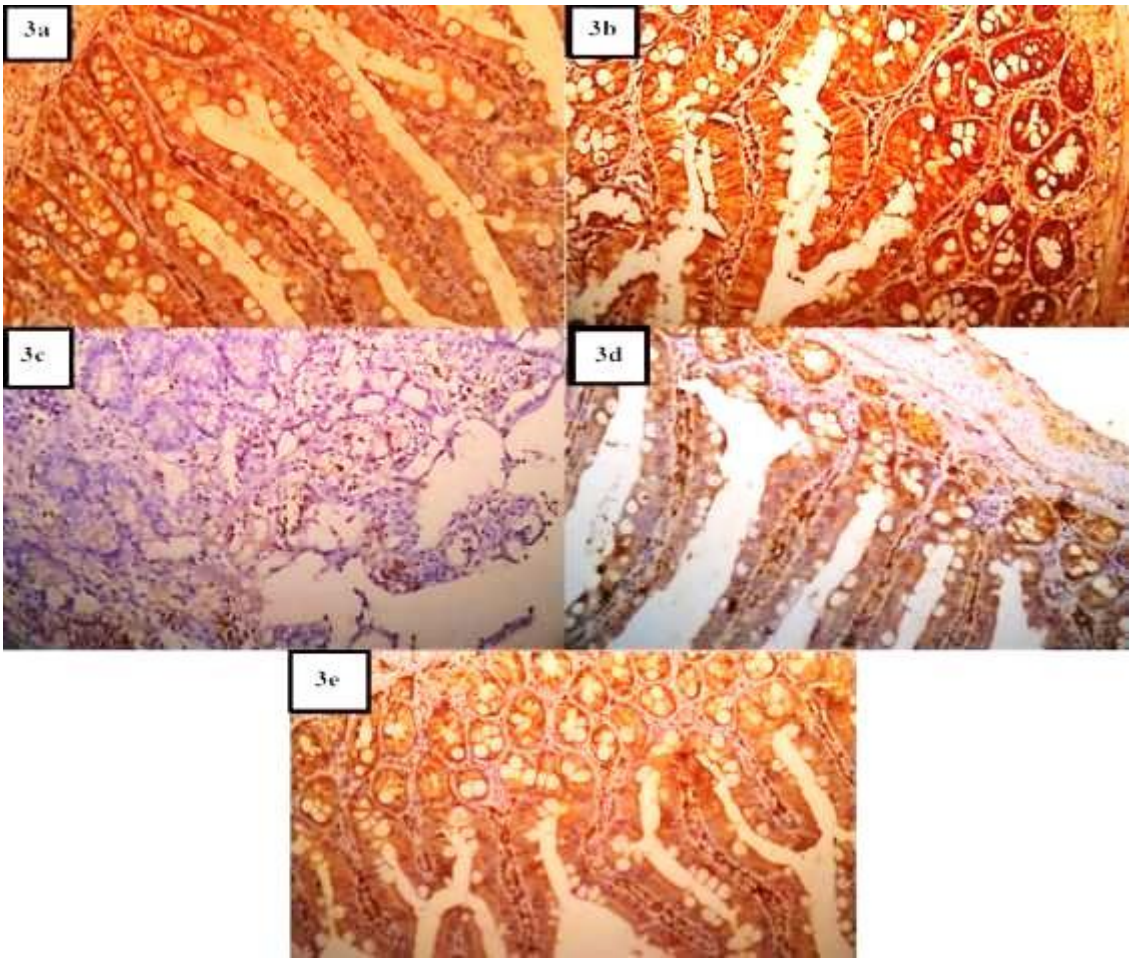


Figure. (3): photomicrographs of KI-67 immunostained jejunal sections revealed that crypt lining cells of group I **(3a)** and group II **(3b)** showing**:** strong positive KI-67 immunoexpression; group III **(3c)** showing very weak immunoreaction; group IV **(3d)** showing moderate immunoexpression; group V **(3e)** showing strong immunoreaction. **(KI-67 X 200)**

Table (3): Statistical analysis of mean values of area % immunoreaction of KI67 ± SD in the studied groups.

Groups / parameter	Control Subgroup Ia	Subgroup IIb (RES H)-treated group	Group III(MTx)-treated group	Group IV (MTx+RES L)-treated group	Group V (MTx+RES H) - treated group
KI-67	42.75± 2.8 c& d	44.54± 4.7 c& d	10.19± 2.8 a,b,d & e	21.82± 3.8 a,b ,c	39.67± 2.2 c

Number of samples = 6 in each group; Data expressed as mean±SD, *: significance ≤ 0.05; One way ANOVA method followed by post-hoc Tukey's test; a: Significance vs Control, b: Significance vs RES H, c: Significance vs MTx, d: Significance vs MTx + RES L, e: Significance vs MTx + RES H.

Immunohistochemical findings:

KI-67 is a nuclear protein present in multiplying cells. The proliferative index of jejunal cells was indicated by the percentage of positive expression of KI-67 immunostaining cells. Control and RES H- treated groups showed strong positive KI-67 immunostaining expression **(Figures 3a &b)**. Whereas, in MTx- treated group, KI-67 antigen positive cells were markedly lowered **(Figure 3c)**. KI-67 expression tended to improve in MTx + RES L- treated group **(Figure 3d)**. However, it was more increased in MTx + RES H- treated group nearly similar to control group **(Figure 3e)**.

Area % of KI-67 immunoreactivity has highly significantly decreased in MTx- treated group in contrast to control and RES H- treated groups. In MTx + RES L treated group, area % of KI-67 immunoreactivity has significantly raised when compared with MTx treated group ($P \leq 0.05$). The area percentage of KI-67 immunoreaction has highly significantly increased in MTx + RES H- treated group when compared with MTx treated group ($P \leq 0.05$), but non-significantly reduced in comparison to control and RES H- treated groups ($p > 0.05$) (Table.3).

IV. DISCUSSION

One of the most commonly used anti-metabolites is methotrexate (MTX) **(Soares et al., 2011**).

In this study, the effect of resveratrol as dose dependent protective agent on the oxidative and jejunal damage that arose as a result of the administration of MTX was examined and assessed.

The main mechanism of MTX induced intestinal toxicity is still unclear. Many scientists explained this toxicity to intestinal oxidants/ antioxidants imbalance. Damage induced by the administration of MTX is characterized by inflammatory cell response **(Soares et al.,2011).** Chemicals resulting from inflammation induct oxidative stress by activation of reactive oxygen species (ROS). This oxidation, in turn, and through cascade of events leads to intra cellular degeneration **(Zhou et al., 2018)**.

Natarajan et al., (2017) suggested that one of the processes through which methotrexate-induced small intestine damage in rats is caused by activation of the mitochondrial apoptotic pathway.

When MTX is administered, the intestinal mucosa exhibits a wide range of biochemical and morphological abnormalities which confirmed in many researches **(Vardi et al.,2008).**

It is reported in this work that levels of MDA, a marker of lipid peroxidation, significantly increased in the MTx group compared to the control and RES groups. These results in accordance with studies of **Arslan et al., (2015**). The rise in MDA level may be attributed to tissue damage and oxygen defense mechanisms which insufficient in the prevention of free radical formation **(Cherian et al., 2019)**. In the MTx +RES treated groups, it was observed a decrease in lipid peroxidation levels compared to that received MTx and the improvement in level of MDA more detected in the MTx + RES H treated group. SOD enzyme plays important role in oxidative stress and is effective in the protective mechanism against oxidative damage (**Fujii et al., 2022)**.

Because methotrexate caused oxidative stress in the current experiment, there was a noticeable decline in the SOD enzyme's activity in the MTx-treated group (**Yulug et al.,2015** & **Gautam et al.,2016).** There was a rise in SOD levels in the MTx+RES treated groups, in a dose-dependent way, resveratrol significantly reduced the intestinal toxicity caused by MTX. Higher levels of the enzyme were seen in the group that received the high dose of resveratrol (**Vardi et al.,2008 and Arslan et al.,2015).**

In this study, jejunal sections of MTx group showed loss of normal configuration of jejunal tissue. Villi were degenerating with inflammatory cell infiltration scattered through. A lot of villi were sloughed and detached. Villar fusion, atrophic villus epithelium were observed. Most crypts were degenerated with cellular debris and necrotic cells. MTX also induces mucosal congestion and bleeding.

These histopathological findings in the MTx group induced intestinal damage were compatible with findings from previous studies **(Vardi et al., 2008; Kesik et al., 2009& Acipayam et al.,2014).**
We noticed also that some crypts showed increase eosinophilic aggregations. Similar studies reported that the intestinal MTX induced damage can be caused by marked inflammation and PNL infiltration **(Arslan et al.,2015**). Because more reactive oxygen radicals, which cause oxidative stress, are produced as a result of increased PNL dissemination. (**Hamada et al., 2013**). In another study, **Yulug et al.,(2015)** added to these findings of intestinal damage in MX group that the crypts have been replaced by cyst like structures.

Since RES plays a role as both a free radical scavenger and also activates antioxidant enzymes, it has been prescribed as a potent antioxidant **(Li ZD et al.,2006 & Tunali et al., 2010**). RES inhibits the peroxidation of membrane lipids **(Heo et al.,2018).** Previous research has shown positive outcomes of RES treatment in a small intestine model with induced ischemia–reperfusion damage (**Karabulut et al.,2006 & Yildiz et al.,2009).**

In our experiment usage of RES as antioxidant after MTx administration reduced the effects of MTx on the small intestine, particularly at the histological level section there was a remarkable improvement in villus structure compared to the MTx group; the appearance of the villous epithelium strcture and number of goblet cells was close to normal, crypts were clearly visible, and this comparable with previous studies of **(Arslan et al.,2015)** who compared between RES and famotidine effect on intestinal mucositis induced by MTX and reported by the end of his study that Famotidine may not be as effective as resveratrol against MTX toxicity in the long term usage, and in another study of **Yulug et al.,(2015)** the RES therapy was accompanied by a noticeable improvement in ileal morphology and an obvious decrease in apoptosis compared to the MTX group. Also agrees with **Zhang et al., (2017)** who surveyed the preventive benefits of resveratrol against radiation-induced intestinal damage, he recorded in his study that resveratrol maintained intestinal cell regeneration.

However, in our experiment the improvement of jujnunal tissue was more obvious in MTx + RES H treated group and this may be attributed to that the high dose of RES presents more protection to intestinal mucosa owing to its anti-inflammatory and antioxidant properties.

Goblet cell migration to the villi is thought to be a sign of cell renewal, and the mucin released by goblet cells is important for the epithelium's protection (**Yeung et al.,2015**). These findings demonstrated that in MTx-treated rats, the number of goblet cells was markedly decreased. However, Rats who were given MTx + RES showed higher goblet cell numbers. Which have increased significantly in MTx + RES H treated group. This matched what had been reported by previous studies of **Yeung et al., (2015) & Yulug et al., (2015).**

Our results reported that MTx induced inhibition of jejunal crypt cellular proliferation but, RES seemed to more influence it in a dose effective manner that the area % immunoreaction of KI-67-stained sections of MTx+ RES H treated group has highly significantly increased in

comparison to MTx treated group and insignificantly diminished when compared to control & RES treated groups.

The current immunohistochemistry finding was also similar to the previous results of **Leitao et al., (2011)** who showed that MTX dramatically decreased the number of cells that were Ki-67 positive, indicating a decrease in proliferation.

Additionally, our outcomes concur with those of **(Acipayam et al.,2014)**, who reported that RES administration increased the Ki- 67-positive rate that MTX injection had decreased. In the intestinal crypts of the rats in the MTX group, the expression of the nuclear protein Ki-67 exhibited a significant decrease.

The enhanced expression of the proliferative marker Ki-67 in the resveratrol-treated group may be a sign that the intestinal damage caused by MTx is healed. **(Zhang et al.,2017)**

Conclusion: this study concluded that Resveratrol could protect jejunum from damaging effect of methotrexate in a dose dependent manner.

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مقارنة تأثيرات الجرعات المختلفة من ريسفيراترول على التأثير السام المستحث بالميثوتريكسات على اللفائفى في الجرذان البيضاء البالغة

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الخلفية: الميثوتريكسات ، دواء سام للخلايا ، يسبب تلفًا شديدًا في الأمعاء الدقيقة ويغير حالتها التأكسدية. الريسفيراترول له نشاط قوي كمضاد للأكسدة ومضاد للالتهابات.
هدفت هذه الدراسة إلى تقييم تأثير ريسفيراترول على السمية المستحثة بالميثوتريكسات للفائفى ومقارنة تأثير الجرعات العالية والمنخفضة من ريسفيراترول في تحسين اللفائفى.
المواد والطرق: تم تقسيم اثنين وأربعين ذكور من الجرذان البيضاء البالغة إلى خمس مجموعات. مجموعة التحكم، ومجموعة RES (حيث قسمت الجرذان الى فئتين تلقت إحداها جرعة منخفضة من ريسفيراترول (RES L) ، وتلقت الأخرى جرعة عالية من ريسفيراترول (RES) عن طريق الفم لمدة 30 يوما)، ومجموعة MTx التي تم فيها إعطاء الجرذان الميثوتريكسات عن طريق الفم بجرعة 5 مجم / كجم يوميًا لمدة ثلاثين يومًا ، ومجموعة MTx + RES L حيث تلقت الجرذان جرعة منخفضة من RES مع جرعة الميثوتريكسات لمدة 30 يومًا ، وتم تلقي جرذان مجموعة MTx + RES H بجرعة عالية من الريسفيراترول مقترنة بجرعة الميثوتريكسات .في النهاية ، تم الحصول على عينات من اللفائفى لفحص الأنسجة ، والكيمياء الحيوية ، والفحص الكيميائي المناعي.
النتائج: تسبب الميثوتريكسات في تلف الخلايا الظهارية والتشفيرية وتسلل خلوي التهابي ملحوظ. كشف التحليل المناعي عن انخفاض التعبير Ki-67، والذي تم تصحيحه بجرعة عالية من ريسفيراترول. الريسفيراترول أيضا أدى إلى تحسين النتائج النسيجية والبيوكيميائية.
الخلاصة: خلصت هذه الدراسة إلى أن الريسفيراترول يمكن أن يحمي اللفائفى من التأثير الضار للميثوتريكسات بطريقة تعتمد على الجرعة.