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Methotrexate Produces Gastrointestinal Stress via Oxidative Stress-caused Acute Physiological Disruptions in Water and Electrolytes Transport in the Mucosal Intestine

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Methotrexate Produces Gastrointestinal Stress via Oxidative Stress-caused Acute Physiological Disruptions in Water and Electrolytes Transport in the Mucosal Intestine

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Abstract
Methotrexate (MTX), a chemotherapeutic agent, is used to treat various types of cancers. MTX was known for its toxic effects, particularly in the gastrointestinal (GI) tract. Consequently, the objective of this present research was to investigate the GI disorders during oxidative stress in rats subjected to oral dose of MTX (100 mg kg^{-1}). Thirty male Wistar rats were equally divided at random into three groups (10 animals in each group): the unexposed group and two groups treated with a single dose of MTX. Acute diarrhea was assessed in rats using the defecation and enteropooling methods. Electrolyte levels in intraluminal fluid were analyzed by flame photometry. Oxidative stress indicators and intracellular mediators were determined in mucosal intestine by colorimetric methods. The MTX treatment of rats caused critical changes in the gastrointestinal functions. Mainly, intensification of the liquid stools and intestinal fluid accumulation as well as perturbation in the electrolyte transport was observed. In addition, MTX has a prooxidant effect, which was indicated by an augmentation of malondialdehyde (MDA) and H_2O_2 generation and a decrease of the enzymatic antioxidants such as SOD, CAT, and GPx. These effects were accompanied with hispathological injury and alteration of lipid metabolism and intracellular mediators such as free iron and calcium. In summary, we found a close association between the gastrointestinal disruptions and the oxidative stress intensity induced by MTX in rats.

Keywords: Methotrexate; Gastrointestinal disorders; Diarrhea; Oxidative stress; Electrolyte transport; Rats.

1. INTRODUCTION

Methotrexate is used widely as a chemotherapeutic agent in the treatment of numerous cancer types. It is also used in the treatment of various inflammatory diseases [1]. The side effects of MTX may include gastrointestinal system dysfunctions and the appearance of nausea, vomiting, and diarrhea [2, 3]. Low dose therapy causes a diverse category of hepatotoxicity, which contains cirrhosis. However, long-term administration of MTX produces anemia. The use of high dose or the chronic exposition to MTX can cause alterations in liver and fibrosis. MTX induces nephrotoxicity and seems to be toxic as well to respiratory and genital organs at very low doses for repeated treatment [4].

Several studies support the contribution of oxidative stress in MTX-produced small-bowel disruption. Reactive oxygen species (ROS) production stimulates the augmentation of the number of leukocytes in the organs, and hence provokes tissue damage through activated neutrocytes [5]. MTX treatment leads to a diminution in methionine synthesis and in the three main antioxidant enzymes (superoxide dismutase, catalase (CAT), and glutathione peroxidase). A deficiency of these parameters caused by MTX may be a reason for elevated ROS. Altered balance between ROS generation and antioxidant defense system provokes the installation of an oxidative stress and could cause numerous pathological states [6]. Therefore, the primary objective of the present consideration was to explore the implication of oxidative stress in water–electrolyte imbalance induced by MTX in the small intestine of rats.

2. MATERIALS AND METHODS

2.1. Different Chemicals Used in Experiment

The diverse chemicals including hydrochloric acid (HCl), trichloroacetic acid (TCA), 2-Thio-barbituric acid (TBA), hydrogen peroxide, ether, and butylated hydroxytoluene (BHT) were obtained from Sigma chemicals Co (Germany). Biochemical kits were purchased from Biomaghreb society (Tunisia). Methotrexate was acquired from Tunisian central pharmacy.

2.2. Experimental Animals

Wistar male rats (200 ± 30 g) were obtained from Tunisian Pharmaceutical Industry. The animals were acclimatized to well-defined conditions (temperature that varies between 19°C and 23°C as well as standard daily light) before the experiments and were fed food with free access to distilled water. The different experiments were conducted based on the National Institute of Health Guidelines for Animal Care and authorized by the Ethics Committee of the University of Tunis.

2.3. Physiological Studies

Experimental procedure described by Dosso *et al*. [7] was used for the evaluation of the effect of MTX treatment on water–electrolyte balance physiology. The day before experiment, animals were fasted for 16 h. Three groups were used for our experiments. Group A were given saline solution (10 ml kg^{-1}), and it served as a control group. Group B and Group C were treated with MTX (100 mg kg^{-1}), and they served as test groups. Animals were observed for defecation for up to 8 h. For the fluid accumulation and electrolyte movement, 2 h later, the animals were euthanized and the small bowel was isolated. The intestinal fluids were collected into graduated containers, and the contents were measured in ml. The intestinal volume decrease was identified [8], and the electrolyte levels obtained after centrifugation process were established using the flame photometry [9]. The various parameters studied were determined after homogenization in phosphate buffered saline (PBS) solution and centrifugation of intestinal mucosal samples. Supernatants were stored at −80°C.

2.4. Histopathological Analysis

The histopathological modifications were treated with hematoxylin and eosin staining according to the method of Behmer *et al*. [10]. Elaborated histological portions were analyzed using optical microscopy (Carl Zeiss, Heidelberg, Germany).

2.5. Oxidative Stress Indicators Assessment

Concisely, the tissue samples were combined with butylated hyroxytoluene and trichloroacetic acid solution (1% BHT dissolved in 20% TCA). After centrifugation, the obtained sample supernatants were combined with 0.5 N hydrochloric acid and 120 mM thiobarbituric acid in 26 mM Tris and heated for 10 min at 80°C. Resulted absorbance was measured at 532 nm [11]. The −SH group quantification was assessed based on Ellman's technique [12]. The results were presented as nmol of SH/mg of proteins. Carbonyl content of proteins was assessed by the method of Levine *et al*. [13], and the results were expressed as µmol carbonyl residues/mg proteins.

2.6. Enzymatic Antioxidants Evaluation

Superoxide dismutase (SOD) activity was measured by the adapted method of Kakkar *et al*. [14], and the data are reported as units (U) of SOD/mg proteins. Catalase activity was determined with the Aebi method [15], and the data were figured as nmol/min/mg protein. Small-intestinal glutathione peroxidase (GPx) action was estimated via Flohé and Günzler design [16]. The data are reported as nmol of GSH/min/mg protein.

2.7. Intracellular Mediator Determination

Mucosal intestine H_2O_2 was determined by the enzymatic method according to Kakinuma *et al*. [17]. In brief, in the presence of peroxidase enzyme, the hydrogen peroxide interferes with 4-amino-antipyrine and phenol, giving a red colored quinoeimine. The optical density was measured at 505 nm, and the data are reported as µmol H_2O_2/mg protein.

Free iron was measured by the Ferrozine method. Shortly, through pH 4.8, whole Fe^{3+} ions are liberated from transferrin and transformed into Fe^{2+} in the presence of the ascorbic acid as reduction agent. The association of Fe^{2+} and Ferrozine leads to the formation of a complex measurable at 560 nm [18].

Calcium was estimated according to Stern and Lewis [19]. In a concise manner, in a basic solution, Ca^{2+} combines with cresolphthalein, which will induce the formation of a colored complex measurable at 570 nm.

These three mediators were made using marketed kits obtained from the Biomaghreb society, Tunisia.

2.8. Serum Lipids Investigation

To estimate the influence of MTX on lipids levels, after the sacrifice, the abdominal vein blood was picked up after the opening of the abdominal cavity. After, the obtained-blood centrifugation at 2000 × g for 10 min, the serum was collected in eppendorf tubes. Triglycerides, total cholesterol, and high density lipoprotein (HDL) cholesterol were determined by commercially available kits from Biomaghreb, Tunisia.

2.9. Statistical Analysis

During statistical analyses, the mean was obtained using the one-way analysis of variance (ANOVA). The data are representative of 10 distinct investigations. Differences were stated as mean ± SEM and designed significant when the values of p were lesser than 0.05.

3. RESULTS

3.1. Oral MTX Induces Physiological Perturbations in Small Bowel

MTX oral administration at the dose of 100 mg kg^{-1} significantly ($p < 0.05$) increased the stool frequency to the animals (0.94 ± 0.06 defecations) when compared with untreated control rats (0.08 ± 0.03 defecations). It also remarkably elevated intraluminal liquid stools (1.00 ± 0.33 ml) when compared to the control group (0.16 ± 0.06 ml). MTX has a reverse effect

Table 1: MTX oral administration (100 mg kg^{-1}) causes physiological disruptions in small intestine in rat.

	Defecations number (8h)	Intraluminal fluid (ml)	Na^+ (mEq/l)	K^+ (mEq/l)
Control (NaCl, 10 mL kg^{-1})	0.08 ± 0.03	0.16 ± 0.06	8.66 ± 1.12	37.26 ± 2.88
MTX (100 mg kg^{-1})	0.94 ± 0.06$^{\#}$	1.00 ± 0.33$^{\#}$	11.56 ± 1.10$^{\#}$	35.04 ± 1.17$^{\#}$

Animals received MTX (100 mg kg^{-1}) and observed defecation up to 8h and enteropooling up to 2h. Results are expressed as mean ± SEM; n = 10 in each group. Data was analyzed by Statview ANOVA. $^{\#}$: $p < 0.05$ compared to NaCl group.

on the electrolytes exchange in the gut. Thus, the Na^+ concentration was augmented (11.56 ± 1.10 mEq/l) compared to the control group (8.66 ± 1.12 mEq/l). However, the K^+ concentration was diminished (35.04 ± 1.17 mEq/l) compared to normal rats (37.26 ± 2.88 mEq/l) (Table 1).

3.2. MTX Induces Histological Damages

After intoxication of the rats with MTX, many histological lesions in the intestinal epithelium were remarked. These alterations were revealed by the crypt disruptions and the epithelial lesions (Figure 1a-b). In this context, these intestinal changes are confirmed by high histological scores (Figure 1c), when compared to the control group.

3.3. MTX Has a Prooxidant Effect

MDA level was increased significantly ($p < 0.05$) following MTX oral administration (47.2 ± 1.34 10^{-3} nmol/mg proteins) as compared to the healthy control group (33.44 ± 0.77 10^{-3} nmol/mg proteins). In addition, protein–carbonyl generation was elevated remarkably in MTX-treated rats (38.12 ± 1.21 μmol/mg proteins) compared to healthy rats (26.42 ± 0.51 μmol/mg proteins). On the other side, in MTX-treated rats, the −SH groups were notably diminished ($p < 0.05$) (Figure 2).

3.4. MTX Provokes a Decline of Antioxidant Biomarkers

Oral administration of MTX produced a marked oxidative stress in the small bowel, which causes an important reduction of antioxidant enzymes, including the SOD, CAT, and GPx activities in MTX-treated rats compared to their control values (Figure 3).

Figure 1: Histological analysis of mucosal intestine after MTX oral administration (100 mg kg^{-1}): (a) demonstrated an intestinal mucosa without alterations, (b) indicated by a destruction of the crypts and ulceration of epithelial, and (c) presented the histological scores for intestinal changes. $^{\#}p < 0.05$ when compared to the control group. Data represent means ± SEM ($n = 10$).

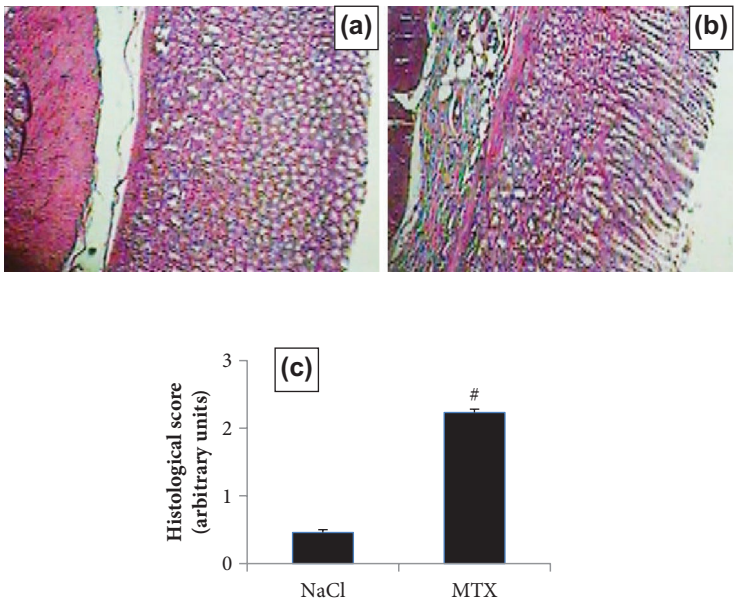


Figure 2: Prooxidant effect of MTX oral administration (100 mg kg^{-1}) on organic macromolecules (lipid and proteins) in rat intestinal mucosa. $^{\#}p < 0.05$ when compared to the control group. Data represent means ± SEM ($n = 10$).

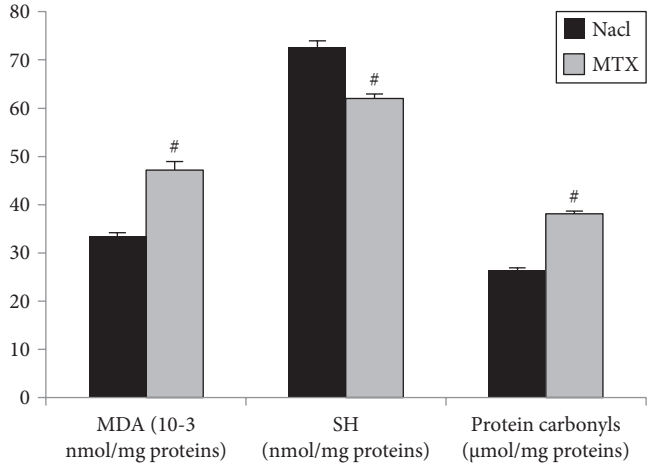
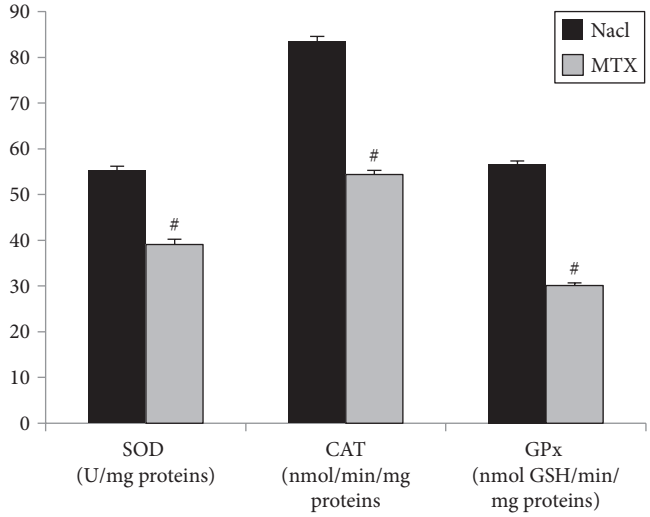


Figure 3: Oral MTX administration (100 mg kg^{-1}) on antioxidant enzymes in small-bowel mucosa of animals. $^{\#}p < 0.05$ when compared to the control group. Data represent means ± SEM ($n = 10$).



3.5. MTX Induces a Perturbation Inintracellular Mediators

To assess the toxic effect of MTX on enterocytes, we evaluated its influence on certain intracellular mediators, such as H_2O_2, free iron, and Ca^{2+}, and the data are indicated in Table 2. The administration of MTX (100 mg kg^{-1}) to rats produces a significant ($p < 0.05$) disorder of these indicators in the intestinal epithelial cells (Table 2).

3.6. MTX Causes a Disturbance in the Lipid Metabolism

Serum lipid contents in MTX-treated rats were also estimated, and the data are provided in Table 3. In this respect, exposure of rats to methotrexate causes a disruption of lipid profile (total cholesterol, HDL cholesterol, and triglycerides). Indeed, MTX induces depletion in TG. However, it provokes an increase in both total cholesterol and HDL cholesterol.

4. DISCUSSION

MTX drug exerts its primary toxic effects against the gastrointestinal epithelium [2]. In this respect, MTX has been shown to lead to hypersecretion and intraluminal fluid accumulation. Both effects were associated with physiological modulations in water

Table 2: MTX oral administration (100 mg kg^{-1}) causes disturbances in intracellular mediator in intestinal mucosa.

	H_2O_2 (µmol/mg proteins)	Free Iron (nmol/mg proteins)	Calcium (10^{-3}nmol /mg proteins)
Control (NaCl, 10mL kg^{-1})	30.24 ± 2.78	21.25 ± 1.47	38 ± 2.83
MTX (100 mg kg^{-1})	41.35 ± 2.46$^{\#}$	31.36 ± 2.33$^{\#}$	54 ± 3.11$^{\#}$

Results are expressed as mean ± SEM; n=10 in each group. Data was analyzed by Statview ANOVA.$^{\#}$: $p < 0.05$ compared to NaCl group.

Table 3: MTX oral administration (100 mg kg^{-1}) causes a disruption in the lipid metabolism.

	Triacylglycerol (mg/dL)	Total cholesterol (mg/dL)	HDL-cholesterol (mg/dL)
Control (NaCl, 10 mL kg^{-1})	77.22 ± 3.26	54.57 ± 3.61	19.43 ± 1.04
MTX (100 mg kg^{-1})	67.11 ± 2.35$^{\#}$	67.22 ± 3.57$^{\#}$	28.28 ± 1.36$^{\#}$

Results are expressed as mean ± SEM; n=10 in each group. Data was analyzed by Statview ANOVA. $^{\#}$: $p < 0.05$ compared to NaCl group.

and electrolyte movements from either side of small intestine, leading to clinically significant diarrhea [20]. These actions can produce a deficiency of liquids and ions, malnutrition, and excessive elimination of water [21, 22].

Methotrexate at a dose of 100 mg kg^{-1} affects the small bowel and causes changes in histology. These damages were represented by destruction and ulceration of epithelial, leading to permeability perturbations and physiological modifications in mucosal intestine. These results agree with previous data, reporting that remarkably deletion in blood cells counts was recognized in the high doses of MTX-intoxicated groups. These modifications in erythrocytes enumeration can be produced by ulceration in the digestive tract inducing, in turn, a gradual blood loss [4].

Very recently, the contribution of oxidative stress to alterations caused by various factors in the digestive tract, essentially the pathophysiology of diarrhea or intestinal hypersecretion, has been demonstrated [23].

Harmfulness investigations by MTX report the role of oxidative stress in producing toxicity on diverse organs [2]. In this respect, acute oral MTX administration provoked an aggression of cells by free radicals in small-bowel mucosa, as determined by oxidation of lipid compounds, protein carbonylation, and depletion of thiol group levels. These changes were caused through a number of paths such as the ROS production. Hence, the generation of ROS leads to the cellular changes and the decrease of the treatment performance [24]. It has been shown that oral way MTX treatment causes oxidative stress and clearly decreases antioxidant enzymes such as SOD, CAT, and GPx, intestinal mucosa, and spinal cord tissues of rats [25]. Thus, the depletion of the antioxidant enzymes activities can be elucidated by their excitation throughout the transformation of molecules with unpaired electrons into metabolites without action, or secondarily with the inhibitory response of MTX on enzyme activities [26].

On the other hand, MTX administration causes an enhanced intracellular mediators production. Indeed, free iron and hydrogen peroxide stimulate the generation of hydroxyl radical through the medium of the Fenton reaction, which causes lipoperoxidation mechanism and disorganization of cellular membranes [27]. The increase of these two mediators following the administration of MTX and the induction of the massive generation of the hydroxyl radical causes the imbalance of Ca^{2+} homeostasis [28].

Finally, our study showed that MTX oral administration has toxicity on lipid metabolism in the dose used. By its association with the digestive tract and adipose tissue, the hepatic tissue possesses a crucial role in numerous conditions of lipid metabolism. MTX low dose therapy has been shown to lead to diverse categories of hepatotoxicity, which includes cirrhosis. However, the use of high-dose MTX or chronic treatment may induce a hepatotoxicity that may lead to progressive fibrosis and cirrhosis [2].

5. CONCLUSION

Oral acute exposure to MTX produced appreciable modulation in intestinal physiological functions leading to significant diarrhea. These changes were accompanied with an intense oxidative stress. Consequently, this study suggests the utilization of habitual examination of oxidative stress indicators in patients who have undergone a chronic MTX treatment.

Author Contributions

Each author contributed equally to this study.

Conflict of Interest

There is no conflict of interest.

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None

References

1. Ozogul B, Kisaoglu A, Turan MI, Altuner D, Sener E, *et al*. The effect of mirtazapine on methotrexate-induced toxicity in rat liver. Sci Asia. 2013; 39:356-62. doi:10.2306/scienceasia1513-1874.2013.39.356.
2. Kumari S. Methotrexate induced hepatotoxicity and its management. Inter J Sci Res. 2016; 5:1477-81.
3. Celik FC, Gocmez M, Bozkurt I, Kaplan K, Kamasak E, *et al*. Neuroprotective effects of carvacrol and pomegranate against methotrexate induced toxicity in rats. Eur Rev Med Pharmacol Sci. 2013; 17:2988-93.
4. Patel NN, Ghodasara DJ, Pandey S, Ghodasara PD, Khorajiya JH, *et al*. Subacute toxicopathological studies of methotrexate in Wistar rats. Veter World. 2014; 7:489-95. doi:10.14202/vetworld.2014.489-495.
5. Abd-Allah OM, Sharaf El-Din AI. The possible protective effect of ginger against intestinal damage induced by methotrexate in rats. Med J Cairo Univ. 2013; 81:1073-84.
6. Cetinkaya A, Bulbuloglu E, Kurutas EB, Kantarceken B. N-acetylcysteine ameliorates methotrexate induced oxidative liver damage in rats. Med Sci Monit. 2006; 12:274-78.
7. Dosso K, N'guessan BB, Bidie AP, Gnangoran BN, Méité S, *et al*. Antidiarrhoeal activity of an ethanol extract of the stem bark of *Piliostigma reticulatum* (Caesalpiniaceae) in rats. Afr J Tradit Complement Altern Med. 2011; 9:242-49.
8. Degu A, Engidawork E, Shibeshi W. Evaluation of the anti-diarrheal activity of the leaf extract of *Croton macrostachyus* Hocsht. ex Del. (Euphorbiaceae) in mice model. BMC Complem Altern Med. 2016; 16:379. doi:10.1186/s12906-016-1357-9.
9. Boominathan R, Devi BP, Dewanjee S, Manda SC. Studies on antidiarrhoeal activity of Ionodiumsuffruticosamging. (Violaceae) extract in rats. Recent Prog Med Plants. 2005; 10:375-80.
10. Behmer OA, Tolosa EMC, Freitas NAG. Manual de tecnicas para histologia normal patologica, EDART—Editora da Universidade de Sao Paulo. Tamboré: Manole (1976), p. 241.
11. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol.1990; 186:421-31.
12. Ellman GE. Tissue sulfhydryl groups. Arch Biochem Bio-phys. 1959; 82:70-77.
13. Levine LR, Garland D, Oliver CN, Amici A, Climent I, *et al*. Determination of carbonyl content inoxidatively modified proteins. Method Enzymol. 1990; 186:464-78.
14. Kakkar P, Das B, Viswanathan PN. Modified spectrophotometric assay of SOD. Indian J Biochem Biophys. 1984; 2:130-32.
15. Aebi H. Catalase. In "Methods in Enzymatic Analysis". Editors—Bergmeyer HU; New York: Academic Press Inc (1974), pp. 673-86.
16. Flohé L, Günzler WA. Assays of glutathione peroxidase. Methods Enzymol. 1984; 105:114-21.
17. Kakinuma K, Yamaguchi T, Kaneda M, Shimada K, Tomita Y, *et al*. A determination of H_2O_2 release by the treatment of human blood polymorphonuclear leukocytes with myristate. J Biochem. 1979; 86:87-95.
18. Leardi A, Caraglia M, Selleri C, Pepe S, Pizzi C, *et al*. Desferrioxamine increases iron depletion and apoptosis induced by ara-C of human myeloid leukaemic cells. Br J Haematol. 1998; 102:746-752.
19. Stern J, Lewis WH. The colorimetric estimation of calcium in serum with o-cresolphthaleincomplexone. Clin Chim Acta. 1957; 2:576-80.
20. Sakai H, Diener M, Gartmann V, Takeguchi N. Eicosanoid-mediated Cl^- secretion induced by the antitumor drug, irinotecan (CPT-11), in the rat colon. Naunyn-Schmiedeberg's Arch Pharmacol. 1995; 351:309-14.
21. Muehlbauer PM, Thorpe D, Davis A, Drabot R, Rawlings BL, *et al*. Putting evidence into practice: evidence-based interventions to prevent, manage, and treat chemotherapy- and radiotherapy-induced diarrhea. Clin J Oncol Nurs. 2009; 13:336-41. doi:10.1188/09.CJON.336-341.
22. Benson AB 3rd, Ajani JA, Catalano RB, Engelking C, Kornblau SM, *et al*. Recommended guidelines for the treatment of cancer treatment-induced diarrhea. J Clin Oncol. 2004; 22:2918-26.
23. Rtibi K, Amri M, Sebai H, Marzouki L. Implication of oxidative stress in small intestine disorders, constipation and diarrhea: a mini review. Rec Adv Biol Med. 2017; 3:66-68. doi:10.18639/RABM.2017.03.457021.
24. Khalil FA, EL-Kirsh AA, Kamel EA, EL-Rahmany NG. Beneficial effect of propolis extract (bee glue) against methotrexate-induced stress in liver and brain of Albino rats. Ind J Med Res Pharmac Sci. 2016; 3:24-34. doi:10.5281/zenodo.54549.
25. Coleshowers CL, Oguntibeju OO, Ukpong M, Truter EJ. Effects of methotrexate on antioxidant enzyme status in a rodent model. Med Tech SA. 2010; 24:5-9.
26. Abdel-Ghaffar FR, Elaimy IA, Dougdoug KA, Nassar HI. Protective and modulatory effects of curcumin and L-carnitine against methotrexate-induced oxidative stress in albino rats. Res J Pharm Bio Chem Sci. 2013; 4:747-54.
27. Sebai H, Jabri MA, Souli A, Rtibi K, Selmi S, *et al*. Antidiarrheal and antioxidant activities of chamomile (*Matricaria recutita*) decoction in rats. J Ethnopharmacol. 2014; 152:327-32. doi:10.1016/j.jep.2014.01.015.
28. Ishii K, Tamaoka A, Takeda T, Ishii K, Iwasaki N, *et al*. Clinical and neurological features of organoarsenic compound (diphenylarsenic acid) intoxication in Kamisu. Japan Rinsho Shink. 2006; 46:768.

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