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PROSPECTIVE VALUE OF FOLIC ACID AND VITAMIN "E" TO MINIMIZE THE TOXIC EFFECTS OF METHOTREXATE THERAPY (ANTINEOPLASTIC DRUG) IN RATS.

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SUMMARY

This study was carried out to investigate the efficacy of folic acid (FA) and/or vitamin E against methotrexate – induced toxicity. Toxic effects of methotrexate (MTX) were studied by the routine toxicity testing. Its effect on body weight gain, hepatic and renal functions were estimated on five experimental groups. The first was intubated with 1.35mg/kg MTX. The second, third and fourth groups were intubated with the same dose of MTX in addition their drinking water was contained 2 mg/l folic acid or 2.5 mg/l vitamin E or both of them, respectively. These treatments were assigned for 3 consecutive days followed by 7 days rest. This regime was repeated five times. The fifth group was served as a control. The obtained results were signs of gastrointestinal manifestations, reduction of body weight gains, elevation of serum aminotransferases activities (ALT,AST and GGT) and increase in the concentrations of serum creatinine and urea. These effects were more prominent in rats administered MTX alone than control and those administered MTX with folic acid and/or vitamin E. However, these changes were significantly much lower in the fourth group (treated with MTX, folic acid and vitamin E) when compared with other MTX-treated groups. Histopathological lesions recorded in liver, kidney and intestines were severe in the group administered MTX only. Administration of folic acid or vitamin E during the periods of MTX therapy reduced these lesions to its moderate form, while both folic acid and vitamin E supplementation relieve it and appeared in a mild form.

In conclusion, MTX therapy has many toxic effects on rats. These effects were minimized by folic acid or vitamin E supplementation. However, administration of both gives the best results for protection. Therefore, we recommended their administration during the course regime of MTX therapy.

INTRODUCTION

Cancer chemotherapy appears to be most effective method against rapidly proliferating cells. It damages the tumor cells as well as the normal host tissues. Application of cancer chemotherapy to animal neoplasia has been lately progressed in clinical veterinary practice. Antifolate compounds have a great value in the treatment of many types of cancer. Methotrexate (MTX) is the most commonly used antifolate for neoplastic disorders. Subsequent studies demonstrated that MTX has a broad-spectrum antineoplastic activity. It may be used as a single or as a part of combined therapy program to enhance its therapeutic effect. It is also used at lower doses for the treatment of non-malignant diseases as psoriasis and rheumatoid arthritis (Boutan et al., 1991 and Long et al., 1996). MTX is a folic acid antagonist specific for the S-phase cell cycle, by inhibiting the enzyme dehydrofolate reductase, which is required in the pathway of DNA, RNA and protein synthesis, leading to deceleration of cell growth. This mechanism acts maximally on cells with the highest rate of growth as in malignant cells (Brody et al., 1993 and Barnhart et al., 2001). The mechanism of MTX toxicity is the same as that of its antineoplastic activity. Methotrexate often has several structural and functional injuries that limit its use or result in unwanted interruptions of its regime therapy and a failure to titrate the optimal level inside the tumor cell, therefore, it loses its therapeutic value as an antineoplastic drug. The recommended MTX regime therapy is always associated with hepatotoxicity, fatal renal or myelotoxic effects. There is circumstantial evidence that MTX therapy contributes to an increased number of bacterial or viral infections due to its immunosuppressive effect (Zeller et al., 1984; Williams et al., 1992 Arias et al., 1993; Bruyn et al., 1995 and Elbadawy et al., 1996).

Folate is the generic descriptor for the folic acid and related compounds exhibiting the biological activity of folic acid. Folonic acid (leucovorin) is the reduced form of folic acid. Folate is reduced *in vivo* enzymatically, first to dihydrofolic acid then to tetrahydrofolate. It acts as an antagonist to MTX and reduces its serious acute toxic effects (Allegra and Boarman, 1990; Buckley et al., 1990 and Cooper, 1996). However, it was ineffective in reducing the severity of MTX-induced pulmonary diseases (Batist and Andrews, 1981, and Pesce et al., 1985).

Vitamin E is recognized as an essential nutrient for human and all other animal species. It has a number of functions other than its biological antioxidant effect. It has an important role in membrane structure, prostaglandin synthesis, blood clotting, disease resistance and immune status. Other functions including its role in electron transport, biosynthesis of DNA within the cells and its protective effect against some toxic elements were also recorded. Vitamin E prevents oral cancer and significantly decreases liver intox-

ication. It has beneficial effects in cardiovascular and neurological disorders (Sakomoto et al., 1991; Garewal, 1993 and Meydani, 1995). Therefore, this study is conducted to estimate whether oral vitamin E supplementation and/or folic acid could be useful for amelioration of MTX-induced toxicity and maintaining its dose intensity known to have an antineoplastic effect and consequently maintaining the tumor exposition time of MTX.

MATERIAL AND METHODS

Drugs:

Methotrexate was obtained from Minapharm Company, Egypt, as a yellow tablet, each containing 2.5mg and soluble in water. Human therapeutic dose as an antineoplastic drug recommended by the company is ranged from 15 – 30 mg / day administered for 3 – 5 days, followed by one week rest. This regime is repeated for 5 – 7 times.

Folic acid was obtained from Nile Company, Egypt, in the form of water–soluble tablets containing 5.1mg. The recommended human dose to reverse the toxic effect of MTX is 15 mg/day (Cooper, 1996).

Vitamin E (alphatocopherol) was obtained from Adwia Company as white water -insoluble powder. It is homogenized in methylcellulose. The recommended human dose is 100 mg/day (Kneckt, 1994).

Doses of these drugs were converted into its equivalent doses for rats and on the basis of their water consumption (Paget and Barnes, 1964).

Animals:

White Spargue-Dawley rats of both sexes were used in this study. They were apparently normal, nearly in the same age (weighing 60 – 90g). The animals were kept under observation two weeks before the start of the treatment, fed on balanced ration and water *ad libitum*. Random classification of these animals was made into five groups, 30 of each. The first group was intubated with 1.35 mg/kg MTX. The second and the third groups were intubated with the same dose of MTX besides their drinking water was contained 2 mg folic acid or 2.5 mg/l of vitamin E, respectively. The fourth group received the same doses of MTX, folic acid and vitamin E. The last group was given tap water and served as control group. These treatments were assigned for 3 consecutive days followed by 7 days of rest. This course was repeated five times.

Methotrexate dosage was selected on the basis of our preliminary study, that the maximum recommended dose used as antineoplastic in rats (2.7 mg/kg) was lethal within 72 hours, therefore we had to choose the minimum therapeutic dose having antineoplastic effect (1.35 mg/kg).

Experiment:

Signs of toxicity on the experimental animals

were assessed daily, while their body weights were recorded periodically after the end of each rest period (every 10 days). At this time, five rats from each group were sacrificed and examined for any macroscopic alterations. Blood samples were collected for serum separation. Collected sera were used for the determination of alanine and aspartate aminotransferases activities (Reitman and Frankel, 1957), gamma glutamyl transferase activity (Persijn and Van der Slik, 1976), creatinine concentration (Henry, 1974) and urea concentration (Patton and Crouch, 1977) using ready made test kits produced by Diamond Diagnostic Company, Egypt and Roch Diagnostic Company, USA. Besides, specimens from liver, kidney and small intestines were collected for the histopathological examination (Lillie, 1984).

Collected data were subjected to analysis of variance using "one – way ANOVA" according to Sendecor and Cochran (1987).

RESULTS AND DISCUSSION

The observed clinical signs in MTX treated groups were mainly in the form of gastrointestinal manifestations including stomatitis, diarrhea, dehydration and loss of appetite, however, water intake was not affected among these groups. Obvious weakness and dullness were also recorded. These signs were attributed to the toxic effects of MTX. The primary cause of lost appetite was resulted from the recorded stomatitis and gastritis. Marked distention of the bowl was recorded during the postmortem inspection. Stomach and intestine were filled with fluids (fig. 1). Diarrhea was due to enteritis as shown by the histopathological examination (figs. 2 and 3). The greater sensitivity of gastrointestinal epithelium is believed to be as a result of the accumulation and persistence of MTX in the intestinal epithelium (Selhub et al., 1983). Similar gastrointestinal manifestations were mentioned during MTX therapy by Allegra et al.,(1986); Furst et al., (1990); Buchbinder et al., (1993); McKendry and Dale (1993) and Sandoval et al., (1995).

Concerning the body weight gain, the statistical data denoted that MTX therapy produced significant decrease in the body weight gains all over the experimental periods when compared with that of the control (table 1, fig. 4). This decrease appeared to be as a result of the accompanied off-food and the persistent diarrhea. It may also be attributed to the recorded failure in the liver functions to anabolize amino acids and protein. Drugs that induce massive hepatic dysfunction usually reflect several hepatic abnormalities such as hypoglycemia and protein calorie malnutrition (Zakim and Boyer, 1982). Poor nutritional status associated with decrease in body weight were also recorded due to long term MTX treatment (Torosian et al., 1988 and Morgan et al., 1990).

signs of MTX toxicity and decreased body weight gains in the four experimental groups were in different degrees. These effects were more prominent in the group administered MTX alone. Administration of folic acid or vitamin E could lower these effects to some extent. However, combined administration of folic acid and vitamin E significantly minimized MTX toxicity when compared with the other MTX-treated groups.

The obtained data revealed that MTX induced hepatotoxicity in all treated groups. This effect was represented by the marked and significant elevation of the activities of liver associated enzymes in the sera (ALT, AST and GGT) as shown in tables 2, 3 and 4 and figures 5,6 and 7. Elevation of serum enzyme activities was directly related to the frequency and the number of doses received. Besides, there were severe characteristic histopathological alterations in the liver (figs 8 and 9). The elevation of transaminase activities in the sera was due to tissue damage particularly in liver, kidney, and heart. Increase of serum GGT activity is considered one of the best indicators for extensive damage of the hepatocytes (Bulle et al., 1990 and Murray et al., 2000). The mechanism of MTX induced hepatotoxicity is referred to the ability of the liver to concentrate MTX in a high level (4-8 times more than that of the plasma). This is due to the presence of folate binding protein in the cytoplasm. Moreover, MTX is converted into polyglutamate form, has intracellular retention, and reduces the polyglutamation of natural folates (Hendel et al., 1985 and McGuire et al., 1986). Also MTX causes increase in the lipid deposition in the liver possibly through limitation of methyl group required for the synthesis of choline (Tuma et al., 1975). This effect is considered a critical factor in the production of fatty liver (Freeman et al., 1977). Several studies demonstrated that hepatotoxicity is the most important potential adverse reaction that can occur during long term therapy with MTX, even when administered in low doses. It has a tendency to cause cirrhosis, fibrosis and fatty degeneration. The speed of onset of liver damage appeared to be dose dependant (Gilbert et al., 1990; Scully et al., 1991; Kremer and Phillips, 1992; Phillips et al., 1992; Kremer and Hamilton, 1995 and Zachariae et al., 1996).

The present results indicated that MTX also induced toxic effects on the renal functions in all treated groups during the treatment periods. The levels of serum creatinine and urea were significantly higher than those of the control (table 5, 6 and figs 10 and 11). Kidneys showed many histopathological lesions (figs 12 and 13). The basis of MTX induced renal toxicity is due to precipitation of both MTX and its less soluble metabolites in the acidic condition. Impaired renal function leads to delay drug clearance and increased risk of MTX crystallization in the renal tubules that damages the tubules (Seideman et al., 1993; Balmer and Valley, 1994; Dorr and Van Hoff, 1994; Kremer

et al., 1995 and Smeland et al., 1996).

Although MTX induced hepatotoxic and nephrotoxic effects in all treated groups, even those supplemented with folic acid or vitamin E, these effects were significantly minimized in the group supplemented with folic acid and vitamin E.

Folic acid and vitamins are attractive targets for antitumor chemotherapy because of their critical role in the synthesis of DNA precursors. Folic acid is a nutritional supplement, essential for normal cell growth and replication. It serves as a carbon donor in the synthesis of purines and thymidine as well as in the remethylation cycle of homocysteine to methionine (Comella et al., 1996). Folic acid antagonizes MTX because of the competitive relationship. It competes with MTX as a substrate for dihydrofolate reductase enzyme allowing resumption of DNA synthesis (Morgan et al., 1990 and Stewart et al., 1991). The statistical analysis of the present results revealed that rats administrated folic acid and MTX showed body weight gains significantly higher than those administered MTX alone. This is due to the action of folic acid to decrease stomatitis and gastrointestinal manifestations induced by MTX. These effects were confirmed by the histopathological examination of the small intestine. Moderate form of enteritis was shown in this group. Improved body weight gains may be due to the role of folic acid as it enters in the decarboxylation and deamination processes improving the utilization of carbohydrate and protein of the food (Morgan et al., 1990). The efficacy of folic acid to reduce gastrointestinal toxicity and oral ulcer during MTX therapy was also recorded by Ortiz et al., (1998).

Folic acid could reduce MTX-induced hepatotoxicity. Significant reductions in the activity of serum liver enzymes (ALT, AST and GGT) were recorded in comparison with those of MTX-treated group. Marked improvement of the histological architecture of the liver was recorded by folic acid administration. Several studies discussed the role of folic acid to reverse the serious acute hepatotoxic effect of MTX. These studies reported that the frequency of serum transaminases elevation was reduced with folic acid supplementation, suggesting that transaminases elevations may be related to hepatofolate depletion and that folic acid is a hepatoprotective (Buckley et al., 1990; Shiroky et al., 1993 and Heathcote, 1996). Folic acid inhibited both systemic and specific hepatic toxicities associated with MTX, by promoting its excretion in the stool (Said and Hollander, 1986 and Priest et al., 1989).

Nephrotoxic effect of MTX was also minimized in the group administered folic acid. The concentrations of serum creatinine and urea were significantly lower than MTX-treated group. The histopathological lesions of the kidney of that group appeared in its moderate form. Folic acid blocks MTX reabsorption through renal tubules, suggest-

ing that it accelerates its excretion and decreases renal toxicity (Allegra, 1990 and Allegra and Boarman, 1990).

Data of several investigators supported the role of folic acid in cancer chemoprevention. They indicated that low tissue level of folate increases the frequency of fragile sites of DNA and increases the risk of DNA to be attacked by carcinogen leading to the potential of chromosomal and oncogen expression. Low level of folate also decreases DNA repair and DNA meythylation (Morgan et al., 1994).

Regarding the protective role played by vitamin E during MTX therapy, the present results denoted that vitamin E also produced a marked effect to reduce the toxicity of MTX. At the present there is no available data about the use of vitamin E or other antioxidants to minimize the toxic effects of MTX therapy or other antineoplastic drugs. However, administration of antioxidants is recommended in cases of chronic inflammations and chronic exposures to pollutants and xenobiotics. There is a statistical correlation between the incidence of diseases and low level of antioxidant nutrients (Meydani, 1995 and Halliwell, 1995). Vitamin E produced its effect by maintaining of membrane integrity of all body cells especially liver and kidney cells. It protects mitochondria, DNA, protein and lipid from destruction, so it helps in the prevention of cancer and other chronic diseases (Kneckt, 1994). Vitamin E prevents the damage of tissues induced by free radicals. These radicals are produced as a result of many chemical exposure and many diseases that lead to the peroxidation of polyunsaturated fatty acids. Free radicals are tumor promoters that inhibit DNA repair, induce chromosomal abnormalities and cause immune dysfunction (Garewal, 1993 and Packer and Fuchs, 1993). Vitamin E appears to be the first line of defense against these radicals by quenching them. It breaks the free radical chain reactions due to its ability to transfer the phenolic hydrogen to peroxyl radical (Murray et al., 2000).

On the basis of these data it could be suggested that the use of vitamin E to reduce MTX toxicity is safe and more convenient because it doesn't compete with MTX, therefore, no expectance of interfering with its action or reducing its efficacy as an antineoplastic drug.

The statistical analysis of variances among MTX-treated groups denoted that the rats that supplemented with both folic acid and vitamin E showed maximum protection level against the different toxic aspects of MTX. Together they decreased the intensity of the clinical signs and increased the body weight gains. Marked improvement of both liver and kidney function tests were recorded. Combined supplementation of folic acid and vitamin E relieved to a great extent the histopathological alterations of the intestine, liver and kidney.

In conclusion, MTX when used in its minimum dose as an antineoplastic drug induced many toxic effects on rats. These effects were minimized by the administration of folic acid or vitamin E; however, administration of both of them gave the best results for the protection. Therefore, their administration during the course regime of MTX therapy is recommended to reduce the frequency and the severity of the associated adverse events.

Table (1): Mean values ± SD of body weight (g) in control and MTX treated groups during the treatment course periods

Group \ Period	First	Second	Third	Fifth	Fifth
Control	99.7 ± 6.2 a	125.9 ± 9.0 a	155.1 ± 8.9 a	180.9 ± 9.2 a	215 ± 10.9 a
MTX	78.5 ± 6.0 b	95.8 ± 6.4 b	113.4 ± 8.4 b	138 ±14.4 b	149.2 ± 9.8 b
MTX + FA.	81 ± 7.0 a,c	99 ± 8.3 b	125 ± 14.8 c	155 ± 16.6 c	178 ± 16.8 c,d
MTX + Vit.E	79.6 ± 9.8 b,c	98.8 ± 12.5 b	122.6 ± 18.7 c	152.4 ± 16.6 c	174 ± 21.6 c
MTX + FA. + Vit.E	81.4 ± 7.5 a,c	105 ± 5.5 c	125 ± 7.2 c	155.4 ± 8.7 c	184.4 ± 10.6 d

Mean values in each column having different scripts are significantly different at $p < 0.05$

Table (2): Mean values ± SD of serum alanine aminotransferase (ALT) activity [u/l] in control and MTX treated groups during the treatment course periods

Group \ Period	First	Second	Third	Fifth	Fifth
Control	36.8 ± 9.6 a	35.5± 1.5 a	34.5 ± 2.7 a	35.9 ± 0.61 a	39.2 ± 4.2 a
MTX	50.9 ± 3.6 b	69.0 ± 4.59 b	93.6 ± 1.5 b	132.1 ± 3.7 b	155.1 ± 2.1 b
MTX + FA.	44.3 ± 1.0 c	67.7 ± 4.5 b,c	86.1 ± 5.6 c	95.1 ± 1.5 c	123.7 ± 2.5 c
MTX + Vit.E	53.0 ± 2.2 b	72.9 ± 4.8 d	84.7 ± 1.5 c	103.6 ± 11.1 d	128.8 ± 5.7 c
MTX + FA. + Vit.E	46.5 ± 2.2 c	65.9 ± 1.1 c,d	79.4 ± 4.1 d	87.1 ± 3.2 e	97.2 ± 2.5 d

Mean values in each column having different scripts are significantly different at $p < 0.05$

Table (3): Mean values ± SD of serum aspartate aminotransferase (AST) activity [u/l] in control and MTX treated groups during the treatment course peri-

Group \ Period	First	Second	Third	Fifth	Fifth
Control	34.2 ± 3.6 a	30.5 ± 3.7 a	34.3 ± 3.4 a	38.8 ± 2.9 a	37.9 ± 4.2 a
MTX	51.1 ± 4.7 b	66.1 ± 4.2 b	80.2 ± 10.1 b	124 ± 10.8 b	156 ± 6.0 b
MTX + FA	43.0 ± 4.2 c	66.0 ± 3.8 b	74.2 ± 2.5 c	74.5 ± 21.3 c	127.5 ± 19.8 c
MTX + Vit.E	47.1 ± 1.3 d	74.4 ± 3.7 c	93.8 ± 6.8 d	116.5 ± 6.8 d	134.9 ± 3.4 d
MTX + FA + Vit.E	48.5 ± 1.9 d	53.3 ± 2.8 d	70.9 ± 2.2 c	82.4 ± 2.5 e	119.6 ± 3.8 e

Mean values in each column having different scripts are significantly differentat $p < 0.05$

Table (4): Mean values ± SD of serum gamma glutamyl transferase (GGT)activity [u/l] in control and MTX treated groups during the treatment course periods

Group \ Period	First	Second	Third	Fifth	Fifth
Control	44.59 ± 4.3 a	48.91 ± 1.96 a	52.6 ± 3.39 a	50.51 ± 5.01 a	52.51 ± 4.12 a
MTX	60.34 ± 1.76 b	70.43 ± 1.17 b	80.96 ± 2.28 b	86.61 ± 7.00 b	143.1 ± 10.8 b
MTX + FA	50.66 ± 5.68 c	68.0 ± 3.37 b,c	66.76 ± 3.01 c	69.53 ± 7.45 c	91.21 ± 4.23 c
MTX + Vit.E	59.34 ± 3.39 b	72.74 ± 2.14 b	84.21 ± 1.34 b	80.49 ± 2.68 d	97.33 ± 1.57 d
MTX + FA + Vit.E	50.17 ± 6.07 c	64.45 ± 2.9 c	72.21 ± 1.36 d	82.93 ± 1.67 d	89.76 ± 6.58 d

Mean values in each column having different scripts are significantly different at $p < 0.05$

Table (5): Mean values ± SD of serum creatinine concentration (g/dl) in control and MTX treated groups during the treatment course periods.

Group \ Period	First	Second	Third	Fifth	Fifth
Control	0.45 ± 0.07 a	0.33 ± 0.023 a	0.33 ± 0.053 a	0.32 ± 0.038 a	0.52 ± 1.23 a
MTX	1.9 ± 0.67 b	2.1 ± 0.49 b	3.2 ± 0.71 b	4.0 ± 0.44 b	4.16 ± 0.25 b
MTX + FA	1.5 ± 0.4 c	1.93 ± 0.23 c	2.7 ± 0.71 c	2.2 ± 0.16 c	3.1 ± 0.54 c
MTX + Vit.E	1.9 ± 0.12 b	3.2 ± 0.26 d	2.7 ± 0.58 b,c	2.9 ± 0./35 d	3.13 ± 0.11 c
MTX + FA + Vit.E	1.6 ± 0.37 c	1.63 ± 0.54 c	0.91 ± 0.84 d	1.5 ± 0.24 e	1.1 ± 0.54 d

Mean values in each column having different scripts are significantly different at $p < 0.05$

Table (6): Mean values ± SD of serum urea concentration (g/dl) in control and MTX treated groups during the treatment course periods.

Group \ Period	First	Second	Third	Fifth	Fifth
Control	27.1 ± 4.3 a	21.2 ± 2.9 a	23.7 ± 4.8 a	23.1 ± 1.8 a	24.2 ± 1.9 a
MTX	62.7 ± 7.4 b	51.6 ± 2.0 b	66.4 ± 2.2 b	62.4 ± 11.0 b	80.6 ± 1.4 b
MTX + FA	33.5 ± 1.4 c	33.6 ± 3.6 c	57.5 ± 9.3 b	43.3 ± 6.4 c	52.6 ± 1.4 c
MTX + Vit.E	34.8 ± 7.5 c	43.4 ± 3.2 d	43.6 ± 3.6 c	54.4 ± 2.5 b	64.6 ± 2.9 d
MTX + FA + Vit.E	22.1 ± 4.6 a	32.6 ± 0.54 c	32.0 ± 2.2 d	41.0 ± 7.4 c	41.9 ± 4.9 e

Mean values in each column having different scripts are significantly different at $p < 0.05$

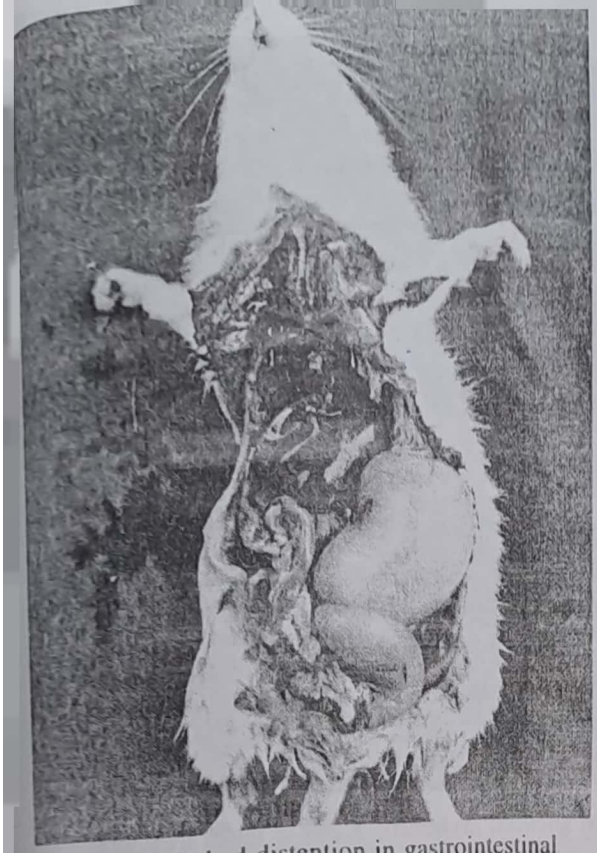

Fig. (1): Marked distention in gastrointestinal bowl in MTX-treated rat.

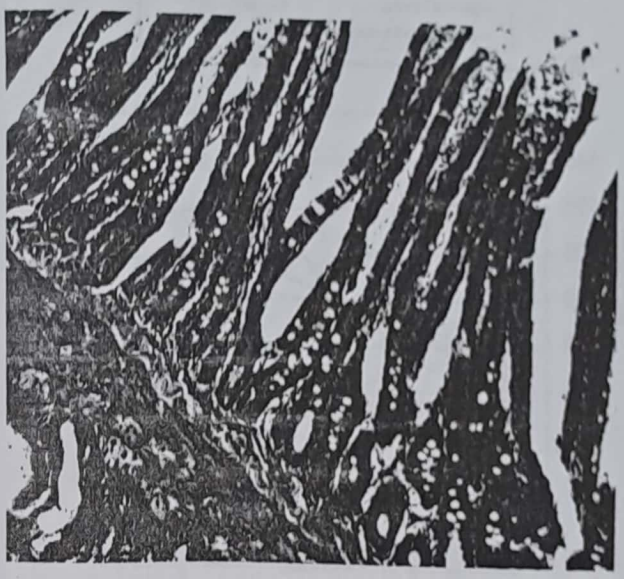

Fig. (3): Small intestine of MTX-treated rat showing hyper-activity of the goblet cells (H&E, X33)

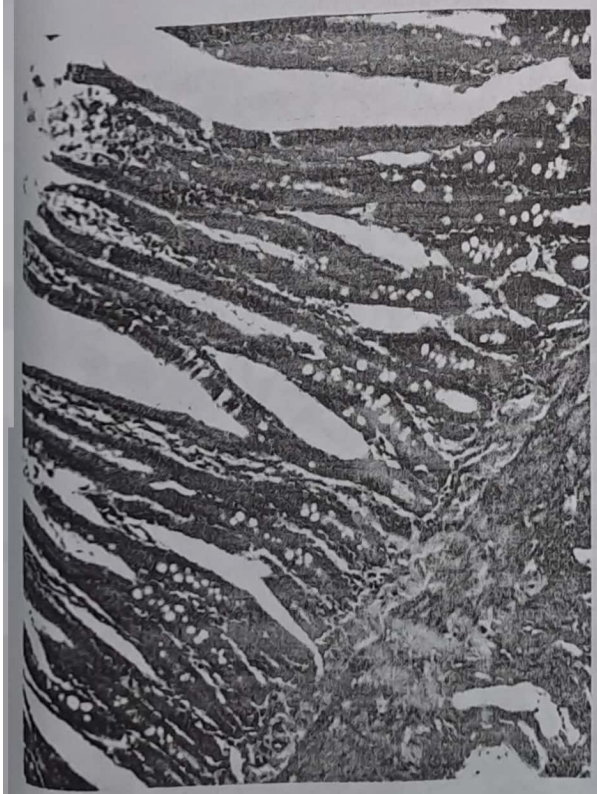

Fig. (2): Small intestine of MTX-treated rat showing inflammatory cells in the lamina propria (H&E, X33).

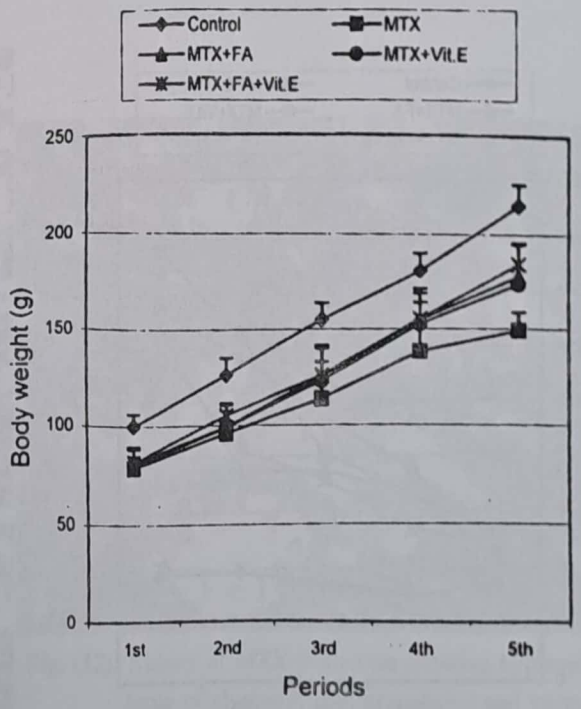

Fig. (4): Mean body weight values in MTX - treated groups

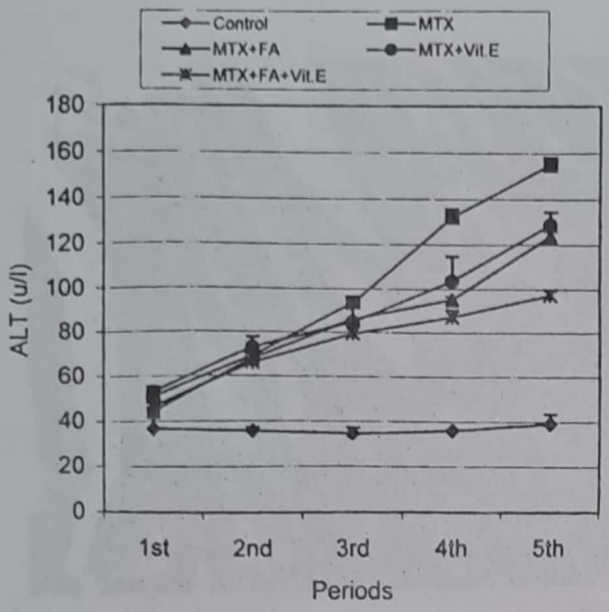

Fig. (5): Mean values of serum ALT activity in MTX - treated groups

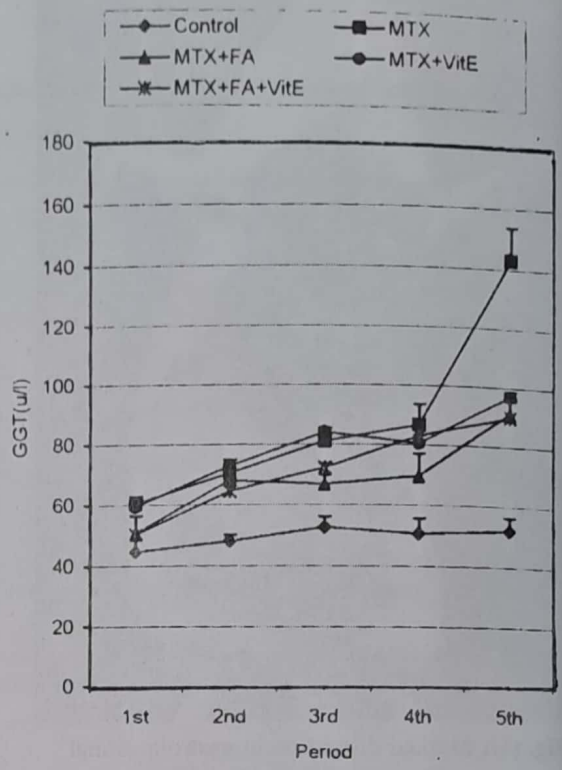

Fig. (7): Mean values of serum GGT activity in MTX - treated groups

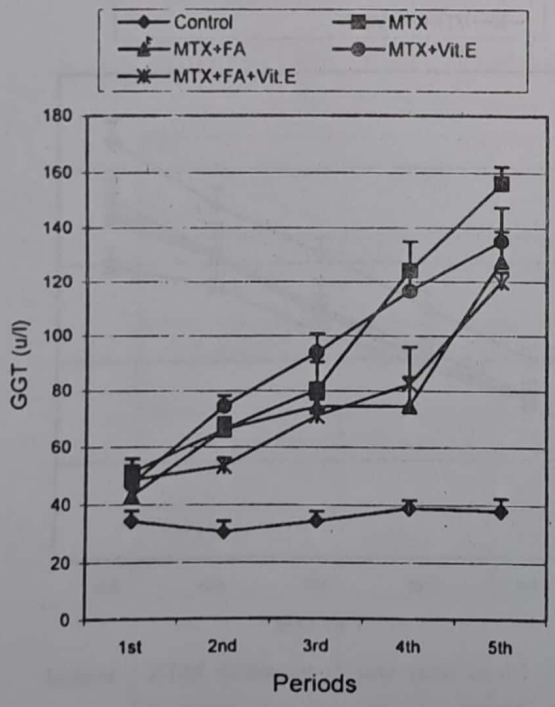

Fig. (6): Mean values of serum AST activity in MTX - treated groups

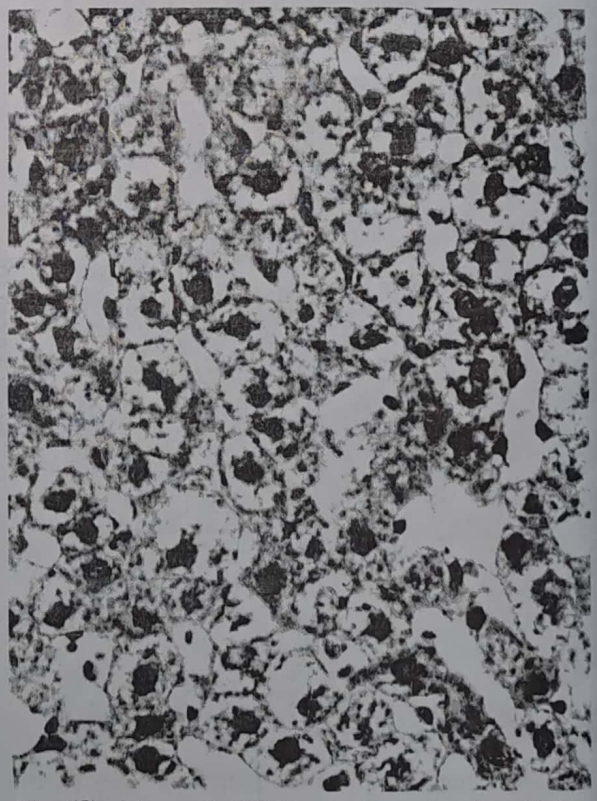

Fig. (8): Liver of MTX-treated rat showing Hydrophobic degeneration, hepatocytes appeared swollen with vacuolation of their cytoplasm (H&E, X132)

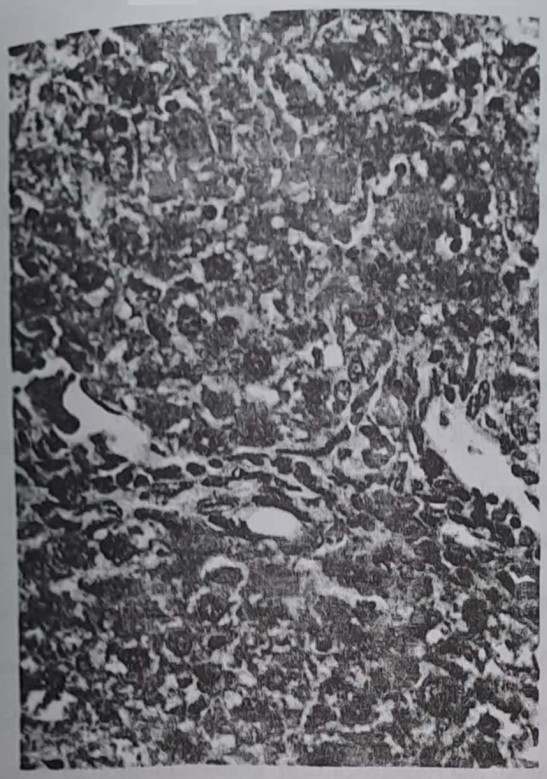

Fig. (9): Liver of MTX-treated rat showing proliferation of bile epithelium with the presence of fibroblasts along the hepatic cord (H&E, X132).

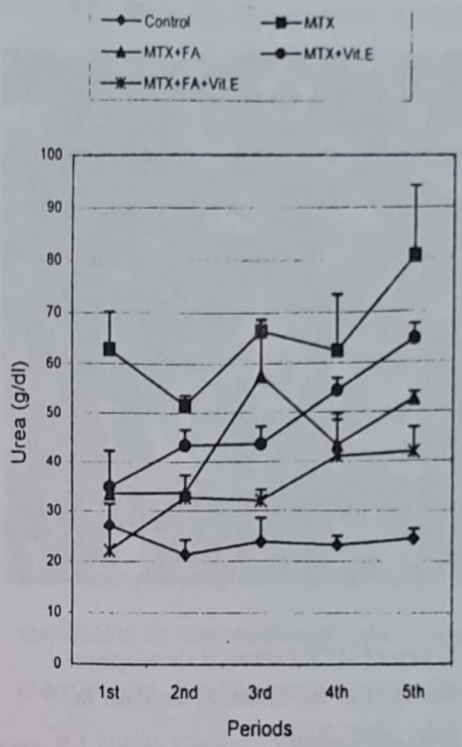

Fig. (11): Mean values of serum concentration in MTX - treated groups

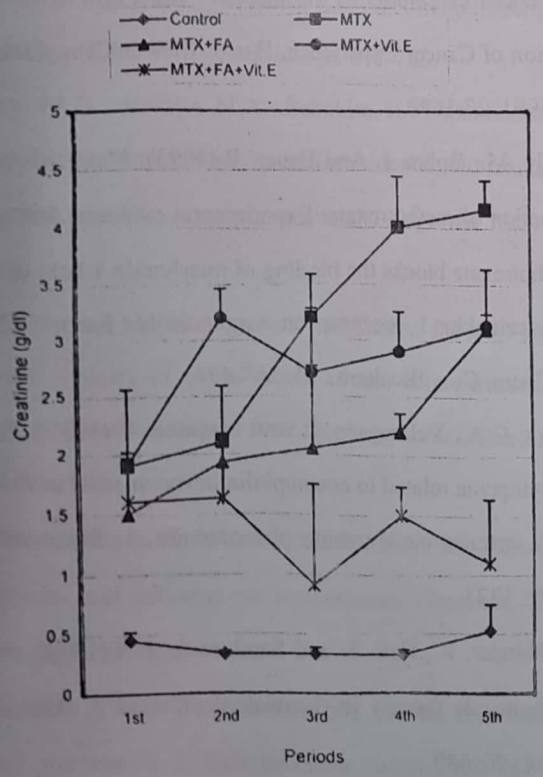

Fig. (10): Mean values of serum creatinine concentration in MTX - treated groups

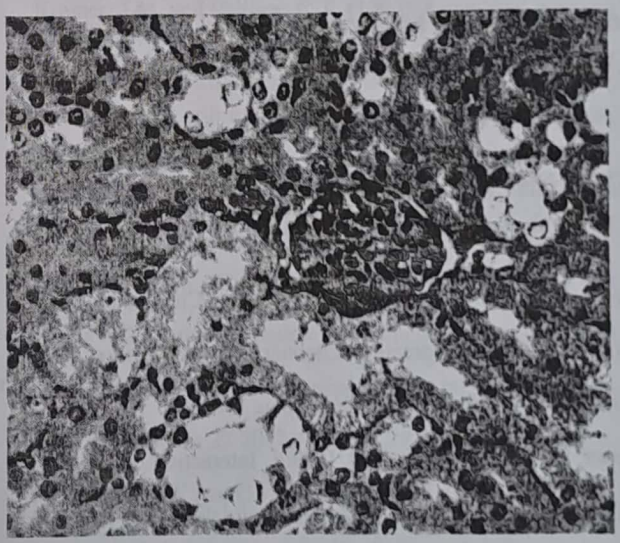

Fig. (12): Kidney of MTX-treated rat showing hypercellularity of glumeruli with granulation and vacuolation of the renal epithelium (H&E, X66).

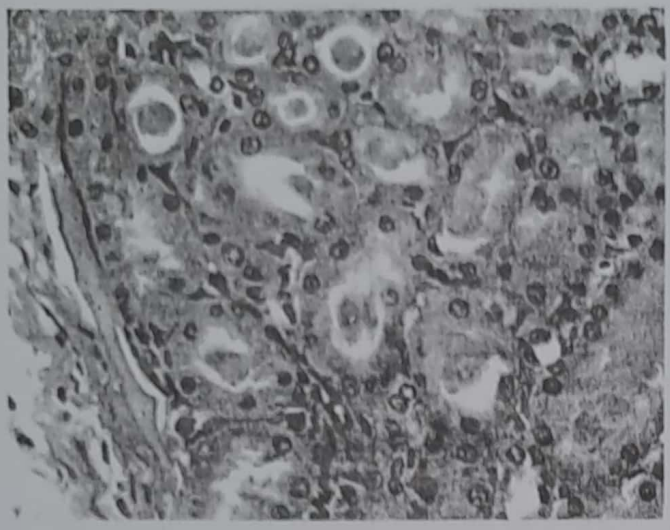

Fig. (13): Kidney of MTX-treated rat showing hyaline casts in the lumen of the renal tubules (H&E, X66).

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