AMELIORATION BY *NIGELLA SATIVA* (L.) OF METHOTREXATE-INDUCED TOXICITY IN MALE ALBINO RATS: A BIOCHEMICAL, HAEMATOLOGICAL AND HISTOLOGICAL STUDY

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There is evidence in the literature of a relationship between dose and response to adjuvant chemotherapy, although published results are conflicting. The use of high dose chemotherapy regimens is limited by the severity of their toxicities. To evaluate tolerability and efficacy of a combination therapy with methotrexate / black seed oil in comparison with methotrexate monotherapy in male albino rats. Methotrexate was administered to male albino rats in an interrupted treatment regimen comparable to proposed human clinical treatment. Animals were dosed orally with methotrexate with/out black seed oil daily for five consecutive days, followed by a seven-days recovery period, and the cycle of dosing and recovery was repeated for a total of four cycles. Changes in the body weight; Hepatic, renal and testicular functions; changes in the haematopoietic system (erythrocytes, leucocytes "total and differential count", platelets, and haemoglobin); and hepatic, renal, gastro-intestinal, testicular as well as brain morphology were all used as parameters to assess methotrexate toxicity. Treatment by methotrexate alone resulted in a) a significant reduction of body weights; b) significant reduction of both the hepatic, renal and testicular functions; c) significant decrease in the haemopoietic parameters; and d) marked alterations in the morphological changes of the liver, kidney, testis and intestine. On the other hand, concomitant treatment with methotrexate and black seed oil resulted in reversibility of methotrexate depression of animal body weights as well as organ functions and morphology. Hence, this study suggests that black seed oil is a pharmacological antidote for methotrexate-induced toxicity, and, therefore, black seed oil in combination with methotrexate may provide a basis whereby more intensive and effective methotrexate therapy may be given.

methotrexate; black seed oil; biochemistry; haematology; histology; albino rats

INTRODUCTION

Nigella sativa (L.) is an annual herbaceous plant growing in countries bordering the Mediterranean Sea (Saad, 1975). The genus Nigella belongs to the botanical family of Ranunculaceae, subphylum Dicotyledons, phylum Angiospermae (Hutchinson, 1989). The name for the genus Nigella was derived from the old Latin word nigellus meaning black, which refers to the black colour of the seed (Ashour, 1988). Nigella has been in use in many Middle Eastern and Far Eastern communities as a natural remedy for over 2000 years, and it was even mentioned in papyruses of the ancient Egyptians 2000–3000 years B.C. (Nassimy, 1984). Nigella is called in several Middle Eastern countries "Habbatul-Barakah" which means "The Seed of Blessing", whereas it is known as "Black Caraway" or "Black Cumin" in Europe (El-Kadi, Kandil, 1987).

Although the immune enhancing effect is a sufficient justification for it to be called "The Seed of Blessing" (Abdullatif et al., 1995), other studies have shown the black seed to have additional healing properties (Hanafy, Hatem, 1991). It possesses the potential of being effective antihypertensive (El-Tahir et al., 1993), antibacterial (Akgul, 1989), antifungal (Anderson, Schiffer, 1962), anti-parasitic (El-Kadery et al., 1997), anti-inflammatory (Houghton et al., 1995), antihistaminic (Chakravarty, 1993), anticoagulant (El-Naggar, El-Dieb, 1992), antiasthmatic (Mahfouz et al., 1965), anti-diabetic (Al-Awadi et al., 1991), anti-tumour agent (Worthen et al., 1998), and a post-coital contraceptive agent (Keshri et al., 1995)

Many chemical compounds have been extracted from *Nigella sativa* (L.), e.g. fixed oil (Mahfouz, El-Dakhakhny, 1960), volatile oil (El-Alfy et al., 1975), alkaloids (Malik et al., 1985), flavinoids (Kirichenko et al., 1971), enzymes (Norbert, 1984), sterols (Salama, 1973), amino acids (Babayan et al., 1978), coumarins (Drozd et al., 1970), ascorbic acid (Ernest, Arthur, 1982), and insecticides (Tipnis et al., 1974). However, it was claimed that the active compounds were mainly found in the volatile oil (El-Dakhakhny, 1963). Furthermore, thymoquinone, the main active constituent of *Nigella sativa*, was also isolated from the volatile oil (El-Alfy et al., 1975).

On the other hand, methotrexate is a cytotoxic agent which has been used with increasing frequency in patients with chronic disease (Baughman, 1998). It is a structural analogue of folic acid that competitively inhibits dihydrofolate reductase, thereby preventing formation of folinic acid and essentially stopping one carbon metabolism. One carbon metabolism is important in the synthesis of methionine, histidine, glycine and purine bases that

are required for the de novo synthesis of DNA (DeSesso, Goeringer, 1992).

Methotrexate is a widely used drug in the treatment of patients with malignant disease (Stark et al., 1989). It has become the disease modifying drug of choice for the treatment of rheumatoid arthritis (Godfrey et al., 1998; Neidel et al., 1998). Methotrexate is also an effective but potentially toxic treatment for psoriasis (Pearce, Wilson, 1996). Recent data indicate that the anti-inflammatory effect of methotrexate is mediated by adenosine. However, methotrexate side-effects can only partly be explained by folate antagonism and may also depend on its action on other related metabolic pathways. The latter include the homocysteine-methionine-polyamine pathway and purine metabolism. Variants in these metabolic routes (i.e. the C677T mutation in the methylene- tetrahydrofolate reductase "MTHFR" gene), may predispose to the development of side-effects (van Ede et al., 1998).

It is likely that all new therapeutic interventions will be used with methotrexate in combination therapy. These combinations may yield real therapeutic advances. The potential for end organ toxicity, opportunistic infection, and malignancy will need to be carefully monitored with long-term meticulously conducted observational studies. Exposure, ease of use, and perceived benefit -to- risk ratio will determine which new agents become most commonly prescribed with methotrexate (Kremer, 1998). Accordingly, the purpose of the present study was to investigate the toxicity of methotrexate administered orally to male albino rats in an interrupted regimen comparable to proposed human therapeutic treatment. Furthermore, the possible protective effect/s of *Nigella sativa* (L.) oil on methotrexate-induced toxicity were also studied.

MATERIAL AND METHODS

Experimental animals

Random-bred, male albino rats weighing approximately 95–142 g were obtained from Animal House Laboratory, National Research Centre, Giza, Egypt. The animals were evaluated prior to initiation of the study to ensure a healthy condition and acclimation to the study environment. Clinically acceptable animals were randomly assigned into 6 groups (10 animals per group), so that there were no statistically significant differences among group body weight means.

Environmental conditions

A total of 60 rats were housed in stainless steel wire mesh cages on a bedding of wood chips (maximum of five animals/cage). They were kept

at an ambient temperature of 25 ± 3 °C, on a light/dark cycle of 12/12 hours, and supplied rat chow and fresh water *ad libitum*.

Test articles and treatments

Methotrexater®, described chemically as N-[4-[[(2,4-diamino-6-petridinyl) methyl] methylamino] benzoyl]-L-glutamic acid, was supplied by LEDERLE LABORATORIES DIVISION, American Cyanamid Company, Pearl River, New York, USA. It is present in white plastic bottles "100 tablets/bottle", each tablet contains an amount of methotrexate sodium equivalent to 2.5 mg of methotrexate and the following inactive ingredients as specified by the supplier: lactose, magnesium stearate and pregelatinized starch; may also contain corn starch. Solutions of methotrexate in 0.9% saline were prepared on the day of administration. The drug was administered orally via gastric tube at 1.8 and 3.6 mg/kg "therapeutic and double therapeutic dose respectively for humans" using a constant dosage volume of 1 ml/kg (Groups III and V, respectively). The negative control animals received an equivalent volume of 0.9% saline (Group I). The rats were dosed once daily for 5 consecutive days followed by a 7-day recovery phase. This cycle of dosing and recovery was repeated for a total of 4 cycles.

Nigellarr® was purchased from Kahira Pharmaceuticals and Chemical Industries Company, Cairo, Egypt. It is present in boxes of 20 capsules (2 strips, each strip contained 10 soft gelatin capsules), each capsule contains 500 mg of extracted oil of *Nigella sativa* L. seeds. The drug was given in a dose of 90 mg/kg orally through a gastric tube in two divided doses. This dosing regimen is similar to that prescribed for human use. The positive control group of animals received an equivalent volume of 45 mg/kg twice daily (Group II), while Groups IV and VI received the same dose of *Nigella sativa* oil simultaneously with the same previously-mentioned doses of methotrexate.

Clinical observations

The animals were observed daily during the 7-week study period for signs of drug toxicity and systemic effects. Complete physical examinations were conducted weekly. Body weights were measured prior to dosing on each dosing day and weekly during recovery periods. Ophthalmic examinations were conducted on all animals pretest and prior to euthanasia.

Necropsies

The total duration of the study was 48 days. On the day 49th, the whole animals were euthanatized for haematology, clinical chemistry and histology studies.

Clinical pathology

Blood for haematological and clinical chemistry determinations was obtained by cardiac puncture from all animals on the day they were scheduled for euthanasia.

Haematology

Blood samples containing EDTA as an anticoagulant were used for the determination of haemoglobin content, erythrocyte count, platelet count and leucocyte count "total and differential".

Clinical chemistries

Clotted blood samples were centrifuged and the serum was removed by aspiration for subsequent determination of the following:

1) Hepatic function tests: Total bilirubin (mg/dl) (Perry et al., 1983); glutamic oxaloacetic transaminase (unit/dl) and glutamic pyruvic transaminase (unit/dl) (Reitman, Frankel, 1957); alkaline phosphatase (unit/l) (Tietz, Rinker, 1983); albumin (mg/dl) (Doumas et al., 1971) and total proteins (mg/dl) (Peters et al., 1982).
2) Renal function tests: urea (mg/dl) (Sampson et al., 1980); creatinine (mg/dl) (Heinegard, Tiderstrom, 1973) and uric acid (mg/dl) (Trivedi et al., 1978).
3) Hormones: testosterone (ng/ml); follicle-stimulating hormone (mIU/ml) and luteinizing hormone (mIU/ml) were estimated using the double antibody test "Diagnostic Products Corporation, Los Angeles, CA, USA" according to the instructions on the package insert of the kit, and using a gamma counter "EMI Nuclear, Enterprise, NE" (Jaffe, Behrman, 1974).

Histology and organ weights

Complete necropsies were performed on all animals. The following organs were weighed in the whole animals: brain, liver, kidneys and testes. Paired organs were weighed separately. Organ-to-body weight ratios were calculated. Tissue samples from the weighed organs as well as the stomach, small and large intestines were taken at necropsy, fixed in 10% formol saline, dehydrated, cleared, embedded in paraffin, and were sectioned at 7 µm. Deparaffinized sections were stained with haematoxylin and eosin as well as Mallory Trichrome stains (Pearse, 1985).

Statistical analysis

The data were analyzed using IBM computer and SPSS-PC4.1 statistical package. Both statistical analysis and tabulation were done according to Knap and Miller (1992). We have used both the paired *t*-test and the analysis of variance (ANOVA) to detect differences in the means between the control group and the other groups of animals. The Newman-Keuls was applied in post-hoc analysis when needed. The level of significance was set at $P < 0.05$.

RESULTS

Clinical observations

Drug-related clinical signs were observed in animals primarily at 40 mg/kg methotrexate only and, in general, appeared during or shortly following the third and fourth cycles of dosing. At 40 mg/kg, alopecia, rhinorrhea, epiphora, and hypoactivity were observed. These signs were rarely observed at lower doses, particularly when simultaneously given with black seed oil. Additional clinical signs observed sporadically during the study included diarrhoea. However, no drug – or treatment – related deaths occurred throughout the whole period of the study.

Body weight and organ weights

There was a significant decrease – in a dose-dependent manner – in the body weights of methotrexate-treated animals when compared with the control groups (Tables I–III). On the other hand, the addition of black seed oil significantly increased the body weights of methotrexate-treated animals and even the positive control group (Tables I and III). However, there was no significant difference in the weight of organs between the control rats and treated ones (Table II).

Clinical chemistry

There was a significant increase – in a dose-dependent manner – in the mean values of serum total bilirubin, SGPT, SGOT and alkaline phosphatase; while the mean values of serum albumin and total proteins were statistically decreased among the methotrexate-treated animals. Similarly, there was also a significant increase – in a dose-dependent manner – in the mean values of serum urea, creatinine and uric acid of methotrexate-treated groups of animals. On the other hand, black seed oil significantly reversed both the hepatic

I. Comparison between the initial and terminal body weights in all studied groups

Groups	Initial body weight (g)		Terminal body weight (g)		Mean difference	S.D. difference	Paired *t*	*p*
	mean	S.D.	mean	S.D.				
Group I	123.300	7.790	216.400	12.076	90.100	4.954	57.511	< 0.05*
Group II	127.100	9.315	230.900	13.470	103.800	4.237	77.464	< 0.05*
Group III	127.000	9.381	185.200	10.141	58.200	1.814	101.484	< 0.05*
Group IV	126.400	11.276	198.700	7.543	72.300	4.001	57.138	< 0.05*
Group V	126.900	11.799	169.200	14.536	42.300	4.668	28.656	< 0.05*
Group VI	127.200	12.674	184.100	18.747	56.900	6.967	25.825	< 0.05*

* = significant

II. Mean values ± S.D. of body as well as organ weights in all studied groups

Group	Initial weight (g)		Terminal weight (g)	
	mean	S.D.	mean	S.D.
Group I	126.300	7.790	216.400	12.076
Group II	127.100	9.315	230.900	13.470
Group III	127.000	9.381	185.200	10.141
Group IV	126.400	11.276	198.700	7.543
Group V	126.900	11.799	169.200	14.536
Group VI	127.200	12.674	184.100	18.747
F	0.013		29.790	
p	> 0.05		< 0.05*	
Group	brain weight (g)		liver weight (g)	
	mean	S.D.	mean	S.D.
Group I	1.494	0.066	6.791	1.098
Group II	1.495	0.057	6.800	0.942
Group III	1.514	0.058	7.115	0.961
Group IV	1.497	0.058	6.832	0.964
Group V	1.536	0.056	7.481	0.929
Group VI	1.509	0.055	7.031	0.918
F	0.758		0.757	
p	> 0.05		> 0.05	

Continuation of Table II

Group	Right kidney weight (g)		Left kidney weight (g)	
	mean	S.D.	mean	S.D.
Group I	0.596	0.040	0.594	0.041
Group II	0.597	0.044	0.595	0.047
Group III	0.609	0.047	0.603	0.046
Group IV	0.603	0.052	0.598	0.052
Group V	0.634	0.043	0.631	0.042
Group VI	0.601	0.043	0.601	0.041
F	0.986		0.941	
p	> 0.05		> 0.05	
Group	right testis weight (g)		left testis weight (g)	
	mean	S.D.	mean	S.D.
Group I	1.001	0.100	0.998	0.100
Group II	0.998	0.108	0.996	0.108
Group III	1.008	0.106	1.005	0.104
Group IV	1.003	0.108	1.002	0.106
Group V	0.993	0.028	0.988	0.027
Group VI	1.002	0.069	1.001	0.064
F	0.324		0.042	
p	> 0.05		> 0.05	

* = significant

III. Mean values ± S.D. of terminal body weight in all studied groups (Newman-Keuls post-hoc test)

Groups	Group I	Group II	Group III	Group IV	Group V	Group VI
Mean	216.400	230.900	185.200	198.700	169.200	184.100
Group I		0.0176*	0.0001*	0.0043	0.0001*	0.0002*
Group II	0.0176*		0.0002*	0.0001*	0.0001*	0.0001*
Group III	0.0001*	0.0002*		0.0265*	0.0245*	0.8533
Group IV	0.0043*	0.0001*	0.0265*		0.0002*	0.0437*
Group V	0.0001*	0.0001*	0.0245*	0.0002*		0.0149*
Group VI	0.0002*	0.0001*	0.8533	0.0437*	0.0149*	

* = significant

IV. Mean values ± S.D. of both liver and kidney function tests in all studied groups

Group	Total proteins (g/dl)		Serum albumim (g/dl)		Total bilirubin (mg/dl)	
	mean	S.D.	mean	S.D.	mean	S.D.
Group I	7.380	0.485	3.467	0.329	0.310	0.110
Group II	7.207	0.648	3.381	0.288	0.340	0.097
Group III	6.065	0.606	2.827	0.475	0.490	0.179
Group IV	7.000	0.859	3.212	0.489	0.380	0.132
Group V	5.263	0.762	2.080	0.544	0.580	0.132
Group VI	6.150	0.787	2.609	0.523	0.470	0.134
F	13.640		13.785		5.957	
p	< 0.5*		< 0.05*		< 0.5*	
Group	SGPT (U/L)		SGOT (U/L)		alkaline phosphatase (U/L)	
	mean	S.D.	mean	S.D.	mean	S.D.
Group I	24.420	3.720	34.100	6.130	81.849	5.529
Group II	31.360	2.819	35.740	5.536	109.830	10.226
Group III	47.460	6.564	60.920	12.065	164.758	10.534
Group IV	34.440	5.156	44.020	6.599	115.540	10.909
Group V	56.410	2.976	71.050	5.580	191.559	11.342
Group VI	45.290	2.966	58.180	5.714	150.628	11.425
F	77.118		41.796		156.911	
p	< 0.5*		< 0.05*		< 0.5*	
Group	serum urea (mg/dl)		serum creatinine (mg/dl)		serum uric acid (mg/dl)	
	mean	S.D.	mean	S.D.	mean	S.D.
Group I	34.900	4.654	0.511	0.072	1.460	0.659
Group II	35.500	4.143	0.602	0.064	1.580	0.666
Group III	65.200	7.146	0.802	0.060	2.260	0.433
Group IV	43.500	5.017	0.663	0.064	1.660	0.682
Group V	85.200	4.185	0.969	0.093	2.660	0.458
Group VI	65.300	5.250	0.841	0.070	2.150	0.460
F	152.772		56.460		6.770	
p	< 0.5*		< 0.05*		< 0.5*	

* = significant

V. Mean values ± S.D. of liver as well as kidney function tests in all studied groups (Newman-Keuls post-hoc test)

Group	Group I	Group II	Group III	Group IV	Group V	Group VI	Group I	Group II	Group III	Group IV	Group V	Group VI	Group I	Group II	Group III	Group IV	Group V	Group VI
	total proteins (g/dl)						serum albumin (g/dl)						total bilirubin (mg/dl)					
Mean	7.380	7.207	6.065	7.000	5.263	6.150	3.467	3.381	2.827	3.212	2.080	2.609	0.310	0.340	0.490	0.380	0.580	0.470
Group I		0.5842	0.0011*	0.4528	0.0001*	0.0015*		0.6723	0.0132*	0.4228*	0.0001*	0.0009*		0.6161	0.0299*	0.4719	0.0006*	0.0455*
Group II	0.5842		0.0035*	0.5129	0.0001*	0.0041*	0.6723		0.0223*	0.4068	0.0001*	0.0020*	0.6161		0.0678	0.5042	0.0017*	0.0828
Group III	0.0011*	0.0035*		0.0120*	0.0137*	0.7879	0.0132*	0.0223*		0.0622	0.0016*	0.2856	0.0299*	0.0678		0.1635	0.1361*	0.7381
Group IV	0.4528	0.5129	0.0120*		0.0002*	0.0092*	0.4228	0.4068	0.0622		0.0002*	0.0118*	0.4719	0.5042	0.1635		0.0077*	0.1361
Group V	0.0001*	0.0001*	0.0137*	0.0002*		0.0181*	0.0001*	0.0001*	0.0016*	0.0002*		0.0116*	0.0006*	0.0017*	0.1361	0.0077*		0.1635
Group VI	0.0015*	0.0041*	0.7879	0.0092*	0.0181*		0.0009*	0.0020*	0.2856	0.0118*	0.0116*		0.0455*	0.0828	0.7381	0.1361	0.1635	
	SGPT (U/L)						SGOT (U/L)						alkaline phosphatase (U/L)					
Mean	24.420	31.360	47.460	34.440	56.410	45.290	34.100	35.740	60.920	44.020	71.050	58.180	81.849	109.830	164.758	115.540	191.559	150.628
Group I		0.0007*	0.0001*	0.0001*	0.0001*	0.0002*		0.6183	0.0001*	0.0104*	0.0001*	0.0002*		0.0001*	0.0001*	0.0001*	0.0001*	0.0002*
Group II	0.0007*		0.0002*	0.1122*	0.0001*	0.0001*	0.6183		0.0002*	0.0144*	0.0032*	0.4062*	0.0001*	0.0002*	0.0001*	0.0002*	0.0001*	0.0032*
Group III	0.0001*	0.0002*		0.0001*	0.0001*	0.2603*	0.0001*	0.0002*		0.0001*	0.0002*	0.0002*	0.0001*	0.2162*	0.0001*	0.0002*	0.0001*	0.0001*
Group IV	0.0001*	0.1122*	0.0001*		0.0002*	0.0001*	0.0104*	0.0144*	0.0001*		0.0002*	0.0008*	0.0001*	0.0001*	0.0002*			
Group V	0.0001*	0.0001*	0.0001*	0.0002*		0.0001*	0.0001*	0.0001*	0.0032*	0.0002*		0.0008*	0.0002*	0.0001*	0.0001*	0.0032*	0.0001*	0.0001*
Group VI	0.0002*	0.0001*	0.2603	0.0001*	0.0001*		0.0002*	0.0001*	0.4062	0.0002*	0.0008*		0.0002*	0.0001*	0.0032*	0.0001*		
	serum urea (mg/dl)						serum creatinine (mg/dl)						serum uric acid (mg/dl)					
Mean	34.900	35.500	65.200	43.500	85.200	65.300	0.511	0.602	0.802	0.663	0.969	0.841	1.460	1.580	2.260	1.660	2.660	2.150
Group I		0.7962	0.0002*	0.0015*	0.0001*	0.0001*		0.0062*	0.0002*	0.0002*	0.0001*	0.0001*		0.6400	0.0223*	0.7143	0.0004*	0.8439*
Group II	0.7962		0.0001*	0.0012*	0.0001*	0.0002*	0.0062*		0.0001*	0.0611	0.0001*	0.0002*	0.6400		0.0482*	0.7551	0.0009*	0.0744
Group III	0.0002*	0.0001*		0.0001*	0.0001*	0.9657	0.0002*	0.0001*		0.0002*	0.0001*	0.2266	0.0223*	0.0482		0.0571	0.1227	0.6680
Group IV	0.0015*	0.0012*	0.0001*		0.0002*	0.0001*	0.0002*	0.0611	0.0002*		0.0002*	0.0001*	0.7143	0.7551	0.0571		0.0015*	0.0601
Group V	0.0001*	0.0001*	0.0001*	0.0002*		0.0001*	0.0001*	0.0001*	0.0001*	0.0002*		0.0003*	0.0004*	0.0009*	0.1227	0.0015*		0.1221
Group VI	0.0001*	0.0002*	0.9657	0.0001*	0.0001*		0.0001*	0.0002	0.2266	0.0001*	0.0003*		0.0439*	0.0744	0.6680	0.0601	0.1221	

* = significant

and renal functions, but not to the initial values of the control groups (Tables IV and V).

Furthermore, there was a significant decrease in the mean values of serum LH – in a dose-dependent manner – among the methotrexate-treated rats when compared with the control ones. On the other hand, black seed oil significantly increased the mean values of serum LH but not to the initial values of the control groups (Tables VI and VII).

Haematology

There was a significant decrease in the total RBCs, WBCs as well as the Hb % – in a dose-dependent manner – among the methotrexate-treated ani-

VI. Mean values ± S.D. of testosterone, L.H. as well as F.S.H. in all studied groups

Groups	Testosterone (ng/ml)		L.H. (mIU/ml)		F.S.H. (mIU/ml)	
	mean	S.D.	mean	S.D.	mean	S.D.
Group I	6.451	2.396	0.560	0.038	25.249	21.387
Group II	6.400	2.344	0.555	0.038	25.030	21.042
Group III	6.119	2.286	0.521	0.051	23.247	20.036
Group IV	6.364	2.056	0.550	0.038	24.894	20.985
Group V	5.918	2.298	0.508	0.036	22.610	21.098
Group VI	6.113	2.284	0.529	0.038	23.733	21.039
F	0.084		2.688		0.027	
p	> 0.05		> 0.05		> 0.05	

* = significant

VII. Mean values ± S.D. of L.H. in all studied groups (Newman-Keuls post-hoc test)

Group	Group I	Group II	Group III	Group IV	Group V	Group VI
Mean	0.560	0.555	0.521	0.550	0.508	0.529
Group I		0.8030	0.2229	0.8489	0.0500*	0.3196
Group II	0.8030		0.2494	0.7689	0.0789	0.3099
Group III	0.2229	0.2494		0.2617	0.4551	0.6899
Group IV	0.8489	0.7689	0.2617		0.1027	0.2426
Group V	0.0500*	0.0789	0.4551	0.1027		0.4857
Group VI	0.3196	0.3099	0.6899	0.2426	0.4857	

* = significant

mals. On the other hand, black seed oil significantly increased these parameters (Tables VIII and IX). However, blood platelets insignificantly decreased among the methotrexate-treated animals (Table VIII). Furthermore, there was no significant difference in the differential leucocytic count between the control rats and the treated ones (Table X).

Histology

GASTRO-INTESTINAL TRACT

Sections from the stomach, ileum and caecum of either the negative control animals (Figs. 1–3), or the positive control animals (Figs. 4–6) showed the normal histological structure of such organs.

VIII. Mean values ± S.D. of haemoglobin, RBCs, WBCs and platelets in all studied groups

Group	Haemoglobin (g%)		RBCs	
	mean	S.D.	mean	S.D.
Group I	15.218	0.941	8.028	0.869
Group II	15.069	0.904	7.972	0.894
Group III	11.618	1.125	6.482	0.759
Group IV	14.845	0.769	7.863	0.938
Group V	8.231	1.944	4.874	0.716
Group VI	11.551	1.254	6.316	0.733
F	53.506		23.544	
p	< 0.05*		< 0.05*	
Group	WBCs		platelets	
	mean	S.D.	mean	S.D.
Group I	12.539	4.088	819.600	220.399
Group II	12.632	4.108	815.700	218.045
Group III	9.862	1.837	791.900	209.248
Group IV	12.451	4.123	810.800	213.993
Group V	7.586	2.610	772.200	223.697
Group VI	11.409	2.569	789.500	222.400
F	3.601		0.071	
p	< 0.05*		> 0.05	

* = significant

On the other hand, methotrexate-treated animals (Groups III and V) showed many histological alterations – in a dose-dependent manner – including widening of the gastric submucosa with marked congestion of its blood vessels (Fig. 7), marked ulcerations of the mucosa of ileum with absence of villi (Fig. 8), and marked lymphocytic infiltration of the mucosa of the caecum with areas of ulcerations (Fig. 9).

IX. Mean values ± S.D. of haemoglobin, RBCs and WBCs in all studied groups (Newman-Keuls post-hoc test)

Group	Group I	Group II	Group III	Group IV	Group V	Group VI
	haemoglobin					
Mean	15.218	15.069	11.618	14.845	8.231	11.551
Group I		0.7857	0.0002*	0.7737	0.0001*	0.0001*
Group II	0.7857		0.0001*	0.6829	0.0001*	0.0002*
Group III	0.0002*	0.0001*		0.0001*	0.0001*	0.9027
Group IV	0.7737	0.6829	0.0001*		0.0002*	0.0001*
Group V	0.0001*	0.0001*	0.0001*	0.0002*		0.0001*
Group VI	0.0001*	0.0002*	0.9027	0.0001*	0.0001*	
	RBCs					
Mean	8.0277	7.9723	6.4816	7.8632	4.8738	6.3162
Group I		0.8810	0.0007*	0.8960	0.0001*	0.0003*
Group II	0.8810		0.0006*	0.7681	0.0001*	0.0004*
Group III	0.0007*	0.0006*		0.0005*	0.0003*	0.6550
Group IV	0.8960	0.7681	0.0005*		0.0002*	0.0004*
Group V	0.0001*	0.0001*	0.0003*	0.0002*		0.0004*
Group VI	0.0003*	0.0004*	0.6550	0.0004*	0.0004*	
	WBCs					
Mean	12.5390	12.6320	9.8620	12.4510	7.5860	11.4090
Group I		0.9508	0.2910	0.9535	0.0141*	0.7326
Group II	0.9508		0.3574	0.9921	0.0169*	0.8467
Group III	0.2910	0.3574		0.2044	0.1348	0.3066
Group IV	0.9535	0.9921	0.2044		0.0106*	0.4900
Group V	0.0141*	0.0169*	0.1348	0.0106*		0.0358*
Group VI	0.7326	0.8467	0.3066	0.4900	0.0358*	

* = significant

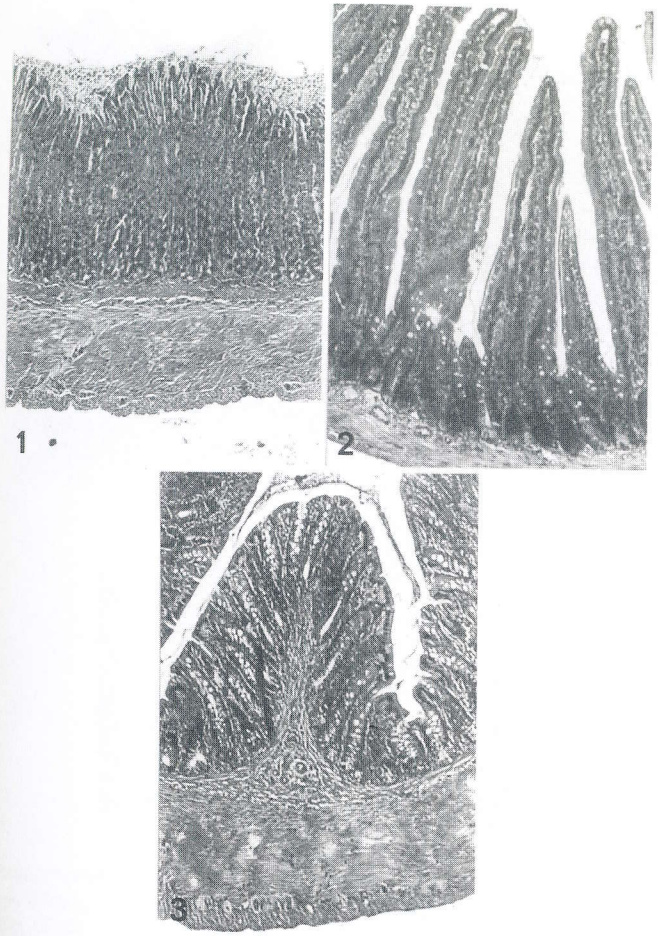
However, on addition of black seed oil to animals (Groups IV and VI), marked improvement in the above-mentioned histological alterations occurred. Although there was also widening of the gastric submucosa, there was only moderate congestion of its blood vessels (Fig.10). Furthermore, there was an enlargement of Peyer's patches to occupy the whole thickness of the ileal wall (Fig. 11). Moreover, the caecum showed an increase in the lymphoid cells of the mucosa (Fig. 12), or marked increase in the goblet cells (Fig. 13).

LIVER

Sections from the liver of either the negative control animals (Figs. 14–15), or the positive control animals (Figs. 16–17) revealed the normal architecture of the liver.

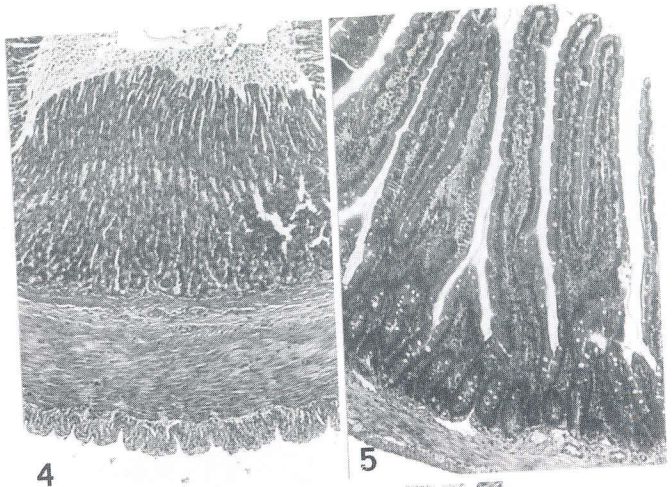
X. Mean values ± S.D. of different leucocytic count in all studied groups

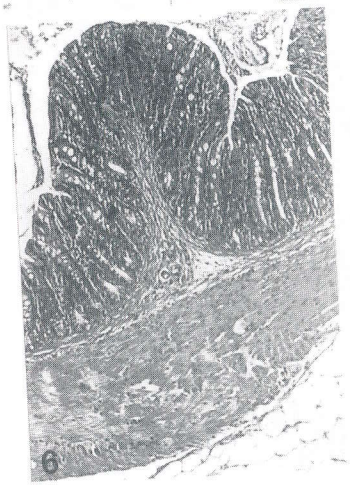
Group	Stab		Segmented		Eosinophils	
	mean	S.D.	mean	S.D.	mean	S.D.
Group I	1.700	0.483	15.300	2.541	1.500	1.080
Group II	1.600	0.516	15.500	2.506	0.900	0.568
Group III	1.500	0.527	14.500	2.635	1.000	0.667
Group IV	1.600	0.516	15.500	2.593	0.900	0.738
Group V	1.500	0.527	14.700	2.541	0.800	0.632
Group VI	1.400	0.516	14.400	2.221	0.900	0.568
F	0.415		0.409		1.200	
p	> 0.05		> 0.05		> 0.05	
Group	basophils		lymphocytes		monocytes	
	mean	S.D.	mean	S.D.	mean	S.D.
Group I	0.000	0.000	78.600	3.893	2.900	0.994
Group II	0.000	0.000	78.900	2.514	3.100	0.876
Group III	0.100	0.316	78.900	4.332	4.000	1.414
Group IV	0.100	0.316	78.600	2.503	3.300	0.949
Group V	0.100	0.316	78.800	3.327	4.300	1.494
Group VI	0.200	0.422	79.000	2.944	4.100	1.370
F	0.711		0.025		2.366	
p	> 0.05		> 0.05		> 0.05	



1–3. Photomicrographs of sections in the stomach, ileum and caecum of Group I animals, i.e. The negative control group, showing:
1. Normal histological structure of the stomach (Hx. & E. x 100)
2. Normal histological structure of the ileum (Hx. & E. x 100)
3. Normal histological structure of the caecum (Hx. & E. x 100)

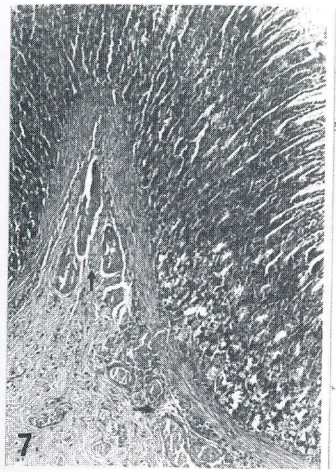
On the other hand, methotrexate-treated animals showed marked histologi - cal alterations – in a dose-dependent manner – including dilated congested portal vein (Figs. 18 and 21), marked lymphocytic infiltration in the portal area (Fig. 19). Furthermore, many binucleated hepatocytes were seen (Fig. 20).

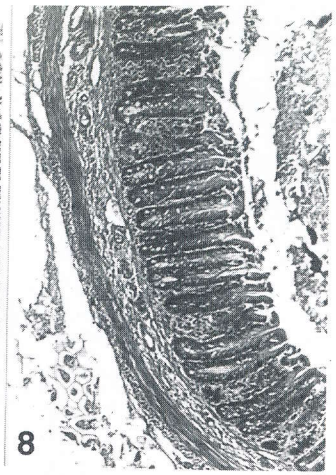


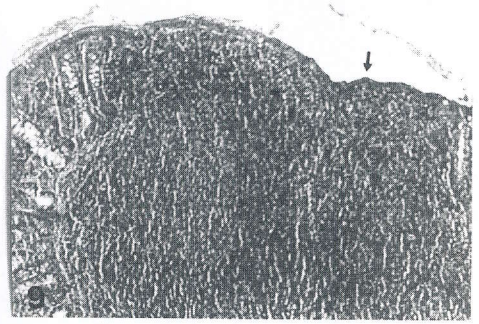


4–6. Photomicrographs of sections in the stomach, ileum and caecum of Group II animals, i.e. The positive control group, showing:
4. Normal histological structure of the stomach (Hx. & E. x 100)
5. Normal histological structure of the ileum (Hx. & E. x 100)
6. Normal histological structure of the caecum. (Hx. & E. x 100)

Degeneration of hepatocytes in the form of vacuolation of cytoplasm, pyknosis of nuclei and fatty degeneration of some cells was also observed (Figs. 20–21). Moreover, intrahepatic haemorrhage (Fig. 20), areas of necrosis (Fig. 18) and marked periportal fibrosis (Fig. 21) were also observed.

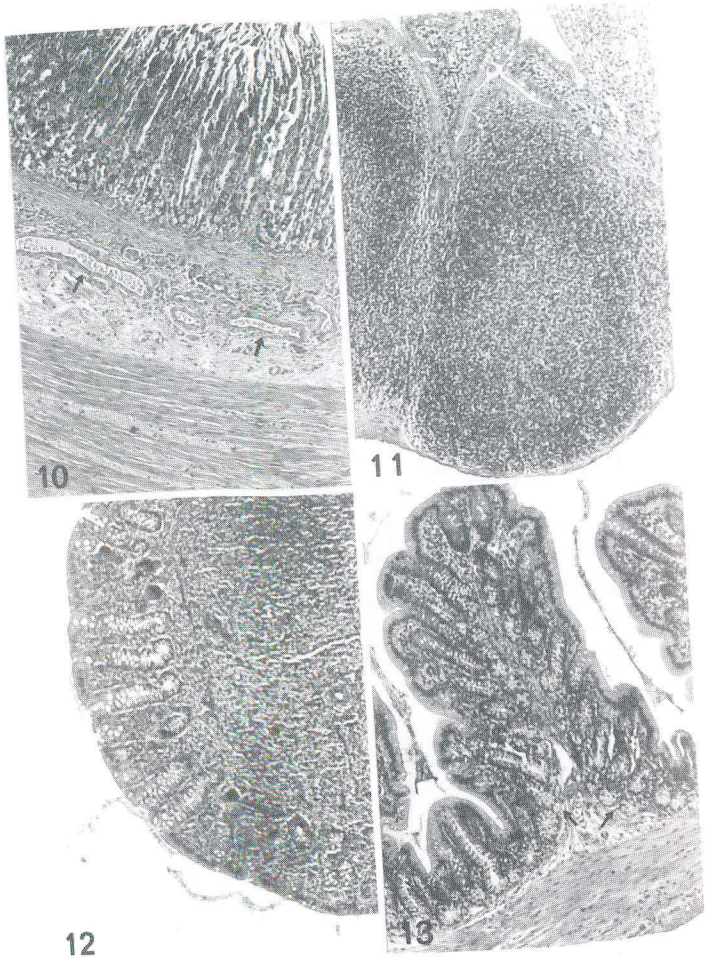




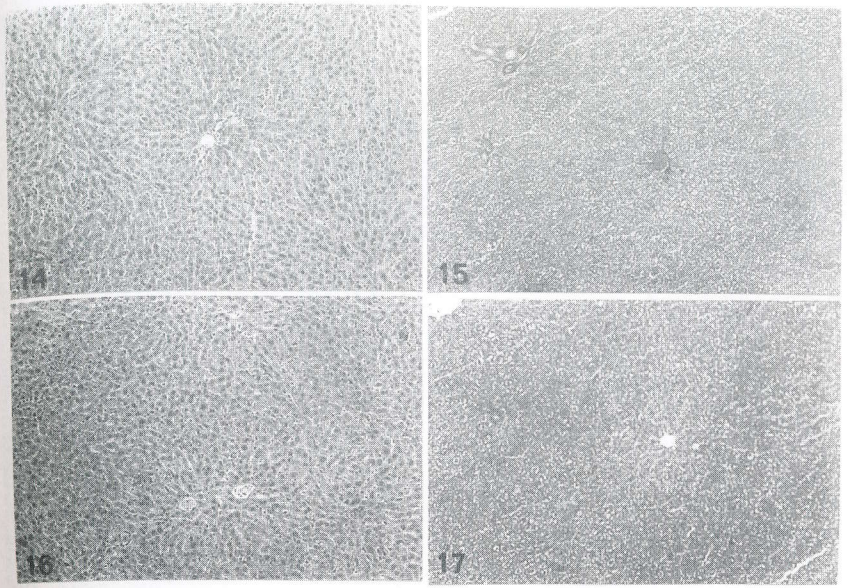


7–9. Photomicrographs of sections in the stomach, ileum and caecum of methotrexate-treated animals, i.e. (Group III – Fig. 7 and Group V – Figs. 8 and 9), showing:
7. Widening of the gastric submucosa with marked congestion of its blood vessels "arrows" (Hx. & E. x 200)
8. Marked ulcerations of the mucosa of ileum with absence of the villi (Hx. & E. x 100)
9. Marked lymphocytic infiltration of the mucosa of the caecum with areas of ulcerations "arrows" (Hx. & E. x 200)

However, on addition of black seed oil to animals, marked improvement in the lymphocytic infiltration (Fig. 22), less congestion in the portal vein (Fig. 23) and less extent periportal fibrosis (Fig. 24) were all observed. However, there was still degeneration of hepatocytes in the form of vacuolation of cytoplasm and pyknosis of nuclei (Figs. 23–24).



10–13. Photomicrographs of sections in the stomach, ileum and caecum of methotrexate / black seed oil-treated animals, i.e. (Group IV – Figs. 10 and 11, Group VI – Figs. 12 and 13), showing:
10. Widening of the gastric submucosa with moderate congestion of its blood vessels "arrows" (Hx. & E. x 200)
11. Marked hypertrophy of Peyer's patches occupying the whole thickness of the wall of the ileum (Hx. & E. x 100)
12. Increase in the lymphoid cells of the mucosa of the caecum (Hx. & E. x 200)
13. Marked increase in the goblet cells of the caecum "arrows"(Hx. & E. x 100)

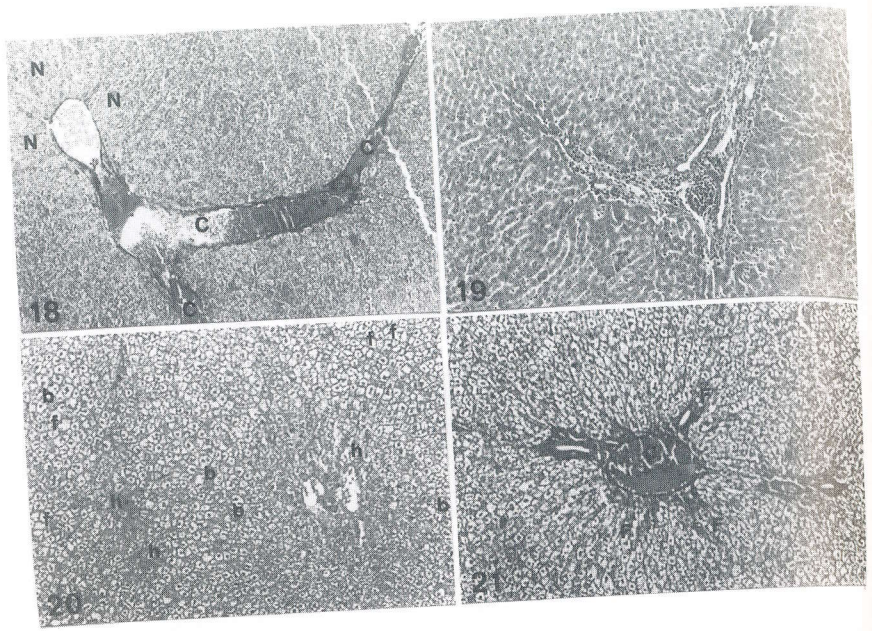


14–17. Photomicrographs of sections in the liver of Groups I and II animals, i.e. The negative and positive control groups respectively (Group I – Figs. 14 and 15, Group II – Figs. 16 and 17), showing:
14. Normal histological structure of the liver (Hx. & E. x 100)
15. Normal collagen content around the central vein and in the portal area (Mallory Trichrome x 100)
16. Normal histological structure of the liver (Hx. & E. x 100)
17. Normal collagen content around the central vein and in the portal area (Mallory Trichrome x 100)

KIDNEY

Sections from the kidney of either the negative control animals (Fig. 25) or the positive control animals (Fig. 26) showed its normal histological structure.

On the other hand, methotrexate-treated rats showed moderate congestion of the cortical blood vessels with mild interstitial haemorrhage, mild interstitial fibrosis, and mild tubular degeneration (Fig. 27). However, the addition of black seed oil markedly improved the extent of fibrosis and degeneration (Figs. 28–29).



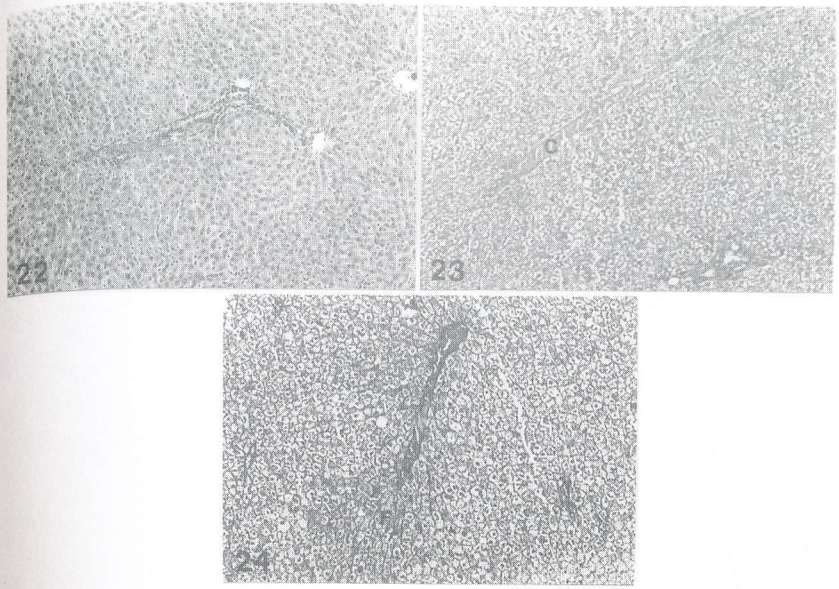
18–21. Photomicrographs of sections in the liver of methotrexate treated rats i.e. (Group III – Figs. 18 and 19, Group V – Figs. 20 and 21), showing:
18. Dilated congested portal veins (C) with an area of necrosis (N) (Hx. & E. x 100)
19. Marked lymphocytic infiltration in the portal area (Hx. & E. x 100)
20. Degenerated hepatocytes "vacuolation of cytoplasm and pyknosis of nuclei". Areas of fatty degeneration (f), many binucleated hepatocytes (b), as well as areas of intrahepatic haemorrhages (h) were also demonstrated (Hx. & E. x 100)
21. Dilated congested portal vein (C), marked periportal fibrosis (F), and degeneration of hepatocytes in the form of vacuolation of cytoplasm and pyknosis of nuclei (Mallory Trichrome x 100)

BRAIN

Examination of brain sections of animals from the different experimental groups showed no apparent changes when compared with the control ones (Figs. 30–33).

TESTIS

Sections from the testes of either the negative control animals (Figs. 34–35) or the positive control animals (Figs. 36–37) showed their normal histological structure.

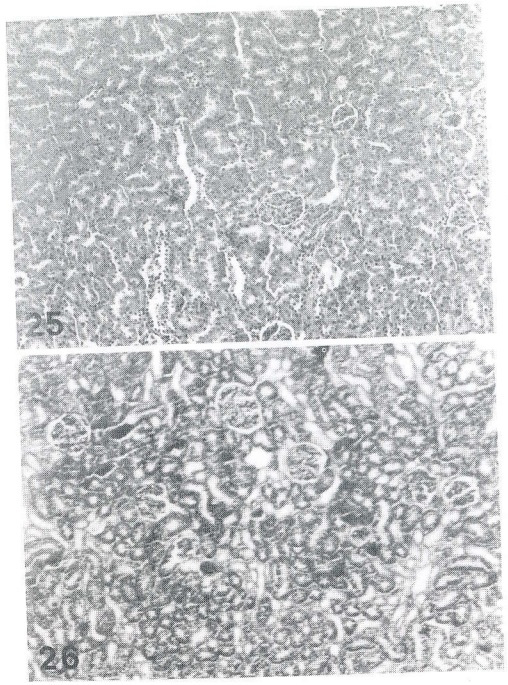


22–24. Photomicrographs of sections in the liver of methotrexate / black seed oil-treated animals i.e. (Group IV – Fig. 22, Group VI – Figs. 23 and 24), showing:
22. Mild lymphocytic infiltration in the portal area (Hx. & E. x 100)
23. Mild congestion of portal veins with moderate degeneration of hepatocytes (Hx. & E. x 100)
24. Mild periportal fibrosis with moderate degeneration of hepatocytes (Mallory Trichrome x 100)

On the other hand, methotrexate-treated animals showed histological alterations – in a dose-dependent manner – including congestion of blood vessels, shrunken degenerated seminiferous tubules with increased interstitial tissue (Figs. 38–39). However, the addition of black seed oil markedly improved these changes (Figs. 40–41).

DISCUSSION

The administration of chemotherapy in clinical situations is limited frequently because of the associated toxicity to host tissues (Rubio et al., 1998). A substantial body of literature accumulating during the past 15 years demonstrates relationship between systemic exposure to various anticancer drugs and their toxic or therapeutic effects (Hon, Evans, 1998). Therefore, preclinical studies have suggested that synergistic anti-tumour toxicity

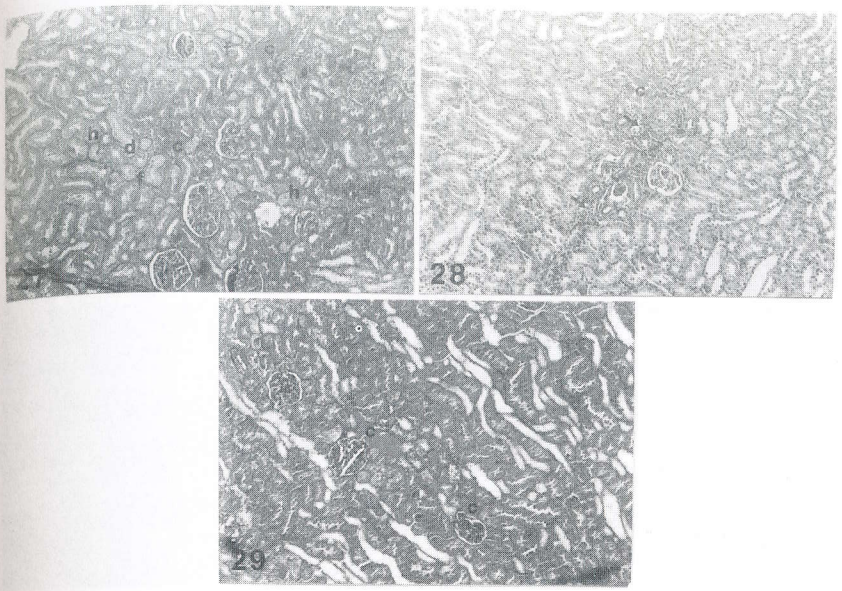


25–26. Photomicrographs of sections in the kidney of Groups I and II animals, i.e. The negative and positive control groups respectively, showing:
25. Normal histological structure of the kidney (Hx. & E. x 100)
26. Normal histological structure of the kidney (Mallory Trichrome x 100)

as well as inhibition of methotrexate-induced toxicity occur when methotrexate is administered prior to, or in combination with other agents, e.g. 5-fluorouracil (Perez et al., 1998), leucovorin (Ortiz et al., 1998), thymidine (Grem et al., 1991), thymidine and purines (Uitendaal et al., 1984), glutamine (Rubio et al., 1998), mifepristone (Perdu et al., 1998), albumin (Wunder et al., 1998), docosahexaenoic acid (Horie et al., 1998), or vanillin and chlorophyllin (Keshava et al., 1998).

On the other hand, interest in herbal medicine is enjoying a renaissance at present (Venugopal, Venugopal, 1995). In our study, we used – for the first time – combination chemotherapy with a regimen of methotrexate and black seed oil. This combination produced a significant improvement in both the biochemical parameters and morphological criteria induced by methotrexate alone in albino rats.

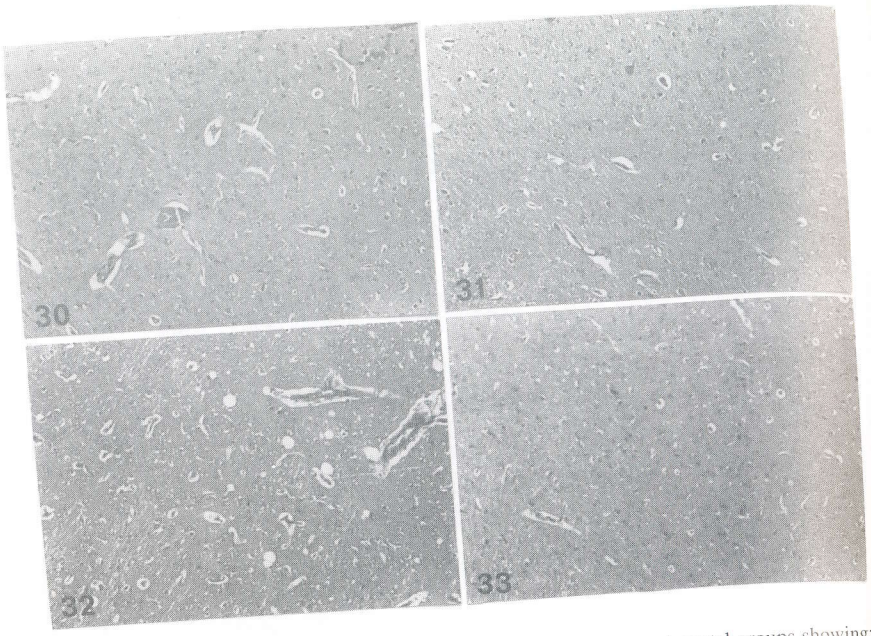
In the present work, methotrexate-treated animals showed significant weight loss or weight gain suppression in an apparent dose-related fashion than the control animals. Similar results were reported by Bowen et al. (1989) and Funk-Archuleta et al. (1997). Chow and Rubin (1997)



27–29. Photomicrographs of sections in the kidney of both methotrexate-treated animals, i.e. (Group III – Fig. 28, Group V – Fig. 27) and methotrexate / black seed oil-treated rats, (Group VI – Fig. 29), showing:
27. Congestion of cortical blood vessels (c), interstitial haemorrhage (h), interstitial fibrosis (f) and tubular degeneration (d) (Mallory Trichrome x 100)
28. Congestion of cortical blood vessels (c) and lymphocytic infiltration (arrows) (Hx. & E. x 100)
29. Congestion of cortical blood vessels (c) (Mallory Trichrome x 100)

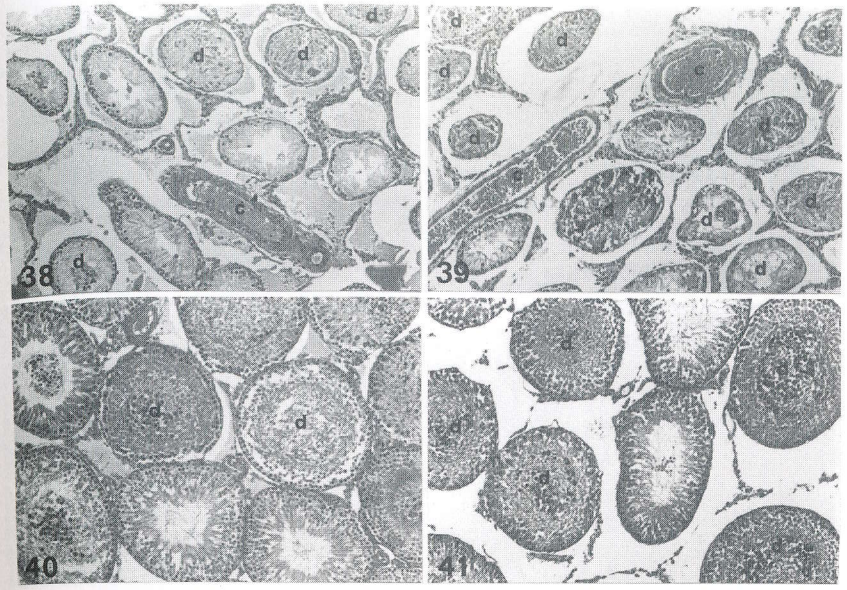
have stated that most cells that retained proliferative capacity after treatment with methotrexate carried random genetic damage that was perpetuated through many divisions of their progeny. However, organ weights of methotrexate-treated rats were increased in also a dose-dependent manner, but differences from the control animals were not statistically significant. On the other hand, animals treated with black seed oil showed a significant increase in their body weights than the negative control animals. Furthermore, organ weights showed a insignificant decrease in rats concomitantly treated with methotrexate and black seed oil than animals treated only by methotrexate, but this decrease did not reach the initial values of the control animals.

Cell cycle-specific chemotherapeutic agents are, of course, expected to cause significant damage to tissues having a relatively high proportion of cells in the proliferative phase at the time of drug exposure (Dethloff,



30–33. Photomicrographs of sections in the brain of the different experimental groups showing:
30. Normal histological structure of a negative control rat (Hx. & E. x 100)
31. Normal histological structure of a positive control rat (Hx. & E. x 100)
32. No histological changes in methotrexate-treated rat's brain compared to the control ones (Hx. & E. x 100)
33. No difference in the brain of methotrexate/black seed oil-treated rat compared to the control ones (Hx. & E. x 100)

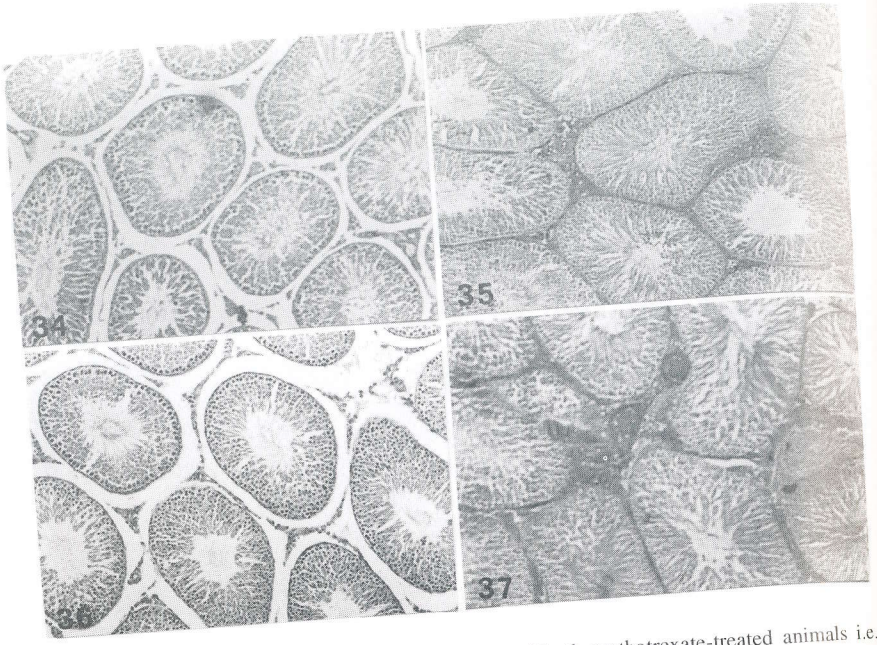
Watkins, 1992). Success of any treatment regimen is dependent upon the elimination of neoplastic cells without significant loss of the regenerative capabilities of normal tissues. The responsiveness of the bone marrow stem cell population to regulatory stimuli, as well as its capacity for both self-renewal and differentiation, underlies its inherent regenerative ability (McCullough, Till, 1971). Almost paradoxically, in a multiple dose anticancer treatment regimen, the wave of proliferation (Vogler et al., 1973) which follows exposure to cell cycle-specific drugs renders affected tissues increasingly sensitive due to the induction of a greater relative proportion of proliferating cells (Meeker, 1967). In the present study, methotrexate also caused significant reductions in both the erythroid and myeloid populations in the peripheral blood. In comparison with our results, many investigators revealed methotrexate-induced significant reductions in RBCs



34–37. Photomicrographs of sections in the testes of Groups I and II animals, i.e. The negative and positive control groups respectively, showing:
34. Normal histological structure of a testis in Group I rats (Hx. & E. x 100)
35. Normal histological structure of the testis in Group I rats (Mallory Trichrome x 200)
36. Normal histological structure of the testis in Group II rats (Hx. & E. x 100)
37. Normal histological structure of the testis in Group II rats (Mallory Trichrome x 200)

(Robbins et al., 1986; Robbins, Bowen, 1988), WBCs (Witjes et al., 1997; Rask et al., 1998), platelets (Viens et al., 1996; Corral et al., 1998), Hb % (Wolfe, Cathey, 1991), and neutrophils (Bjarnason et al., 1998; Ciesielski et al., 1998). Other investigators reported methotrexate-induced significant increase in monocytes (Seitz et al., 1998). Blau et al. (1996) have suggested that the mechanism by which folate analogues exerted their haematological toxicity was through the depletion of relatively mature, non-clonogenic precursor cells, and not by killing progenitors. This information was relevant to the use of dihydrofolate reductase in gene therapy protocols, and suggested that folate analogues were poorly suited agents for selection on the level of clonogenic progenitor cells *in vivo*.

On the other hand, the addition of black seed oil – in our study – significantly reversed the whole previously-mentioned haematological parameters, but not to the initial values of the control groups. In comparison with our



38–41. Photomicrographs of sections in the testes of both methotrexate-treated animals i.e. (Group III – Fig. 38, Group V – Fig. 39) and methotrexate / black seed oil-treated animals (Group IV – Fig. 40, Group VI – Fig. 41), showing:
38. Moderate congestion of blood vessels (c), moderately shrunken seminiferous tubules with few degenerated ones (d), and moderate increase in the interstitial spaces (Hx. & E. x 100)
39. Marked congestion of blood vessels (c), markedly shrunken seminiferous tubules with numerous degenerated ones as well as marked increase in the interstitial spaces (Hx. & E. x 100)
40. Few degenerated seminiferous tubules (d) (Hx. & E. x 100)
41. Mildly shrunken seminiferous tubules with many degenerated ones (d) as well as mild increase in the interstitial spaces (Hx. & E. x 100)

results, Nair et al. (1991) and El-Daly (1998) have stated that an extract of *Nigella sativa* seed tended to protect from cisplatin-induced falls in haemoglobin levels and leucocyte count in mice and rats respectively. Furthermore, Haq et al. (1995) have reported that a stimulatory effect of *Nigella sativa* was noticed on the lymphocyte response to pooled allogenic cells.

Careful attention to dose schedules and the use of leucovorin rescue therapy has essentially eliminated the occurrence of severe and often fatal intestinal toxicity observed previously during early clinical use of folic acid antagonists (Pinkerton, Milla, 1984), but malabsorption syndromes remain a significant concern in humans (Lewis et al., 1982). In the present

study, the observed gastrointestinal lesions were typical for the anti-neoplastic agents, and were in accordance with the lesions described by Warden et al. (1997) and Nakamaru et al. (1998). Furthermore, ultrastructural examination of the small intestinal cells of methotrexate-treated mice showed a marked increase in the size of their mitochondria. The mitochondria were surrounded by channels of rough endoplasmic reticulum; and the cytoplasm contained long, winding channels of smooth endoplasmic reticulum, vacuoles, and myelin figures (Bessler et al., 1996). Fluid retention in the small intestine due to administration of cytotoxic drugs was suggested by the authors to be a possible mechanism for distention of the mitochondria. On the other hand, black seed oil – in our study – markedly improved the morphological architecture of the small intestine, but not to the initial control figures.

Aside from the expected toxicity to rapidly proliferating tissues, such as the gastrointestinal tract and the bone marrow, the major side effects of chronic methotrexate administration in humans is hepatotoxicity (Epstein, Croft, 1969; Dahl et al., 1971). The American College of Rheumatology convened a committee to assess the risks of development of clinically significant liver disease (CSLD) during methotrexate treatment, to evaluate the risk and role of surveillance liver biopsies, and to provide recommendations about monitoring patients for liver toxicity. The committee recommends obtaining liver blood tests (alanine aminotransferase "ALT", aspartate aminotransferase "AST", alkaline phosphatase, albumin and bilirubin), hepatitis B and C serologic studies, and other standard tests including complete blood cell count and serum creatinine tests prior to starting treatment with methotrexate (Erickson et al., 1995).

In our study, the liver of animals receiving black seed oil alone showed neither biochemical nor histological differences from those of the negative control group. On the other hand, there was a significant increase – in a dose-dependent manner – in the mean values of serum total bilirubin, SGPT, SGOT, and alkaline phosphatase enzymes; while total proteins and albumin were significantly decreased in methotrexate-treated rats. Similar results were obtained by Skeith et al. (1994) and Beyeler et al. (1997). However, some investigators have reported no significant increase in the hepatic function tests (Rau et al., 1998); while other investigators have found a slight increase in such biochemical parameters (Wallace et al., 1989). The reasons for such divergent observations of these results may result from the different species used. Furthermore, the different dose schedules, route of administration as well as duration of intake and recovery periods of methotrexate used in the present study can also possibly be responsible for the different results.

In the present study, methotrexate induced histological alterations in the liver in also a dose-dependent manner. The histological lesions are non-specific; consisting of fatty change, nuclear pleomorphism, hepatocyte necrosis, severe congestion of portal veins, intrahepatic haemorrhage, portal chronic inflammatory infiltrate, and fibrosis. The mechanism of liver injury is poorly understood; intracellular accumulation of methotrexate polyglutamate and consequent folate depletion are suspected to play a role (Kevat et al., 1988). Furthermore, because choline supplementation affords partial to complete protection, methotrexate hepato-toxicity appears to be mediated through a reduced folate pool and the depletion of S-adenosylmethionine. The subsequent decrease in choline and phosphatidylcholine synthesis results in the hepatocellular fatty degeneration and necrosis (Freeman-Narrod et al., 1977a, b). In accordance with our results, many investigators revealed numerous histological alterations in the liver due to treatment by methotrexate, e.g. fatty change (Lanse et al., 1985), nuclear pleomorphism (Szanto et al., 1987), hepatocyte necrosis (Kujala et al., 1990), intra-hepatic haemorrhage (Cash et al., 1992), chronic inflammatory infiltrate (Aponte, Petrelli, 1988), and fibrosis (Beyeler et al., 1997).

On the other hand, the simultaneous use of black seed oil – in the present study – resulted in an improvement of both the biochemical parameters and histological changes in the liver of methotrexate-treated animals, but not to the initial figures of the control groups. Similar results were obtained by El-Daly (1998) on cisplatin-induced toxicity in rats.

Similar to the liver, the kidneys of animals treated by black seed oil alone did not show any biochemical or histological differences from those of the negative control group. On the other hand, there was a significant increase – in a dose-dependent manner – in the mean values of serum urea, creatinine and uric acid among the methotrexate-treated animals. Similar results were obtained by Grem et al. (1991) and Koch-Nogueira et al. (1998). However, treatment with low-dose methotrexate produced no significant changes in renal function of either the human patients (Wiland et al., 1997), or the experimental animals (Ermens et al., 1989). The mechanisms controlling the renal retention and urinary output of methotrexate and folates were studied by Deutsch and Kolhouse (1989). They have stated that 125I-labelled folic acid administered intravenously was shown to be retained in the kidney through a system that could be inhibited by either folic acid or 5-methyltetrahydrofolate. Methotrexate also inhibited this system but required concentrations 50 to 100-fold greater than that required for folic acid and 5-methyltetrahydrofolate. Extracts from solubilized kidneys were shown to contain a folate binder with the same relative affinities for folates and methotrexate as the *in vivo* system. Methotrexate was shown to cause an

increase in the urinary output of endogenous folates in rats when administered as equivalent doses to those used in treating human disease. Conversely, "3H"-methotrexate administered intravenously was shown to be retained in the kidney through an additional system that could be inhibited by unlabelled methotrexate, but not by folates. Furthermore, Statkevich et al. (1993) have reported that interaction studies indicated that there was secretory inhibition of methotraxate as evidenced by a decrease in both excretion ratio and tubular clearance at 25 µg/ml of methotrexate.

In the present study, methotrexate induced histological alterations in the kidney in also a dose-dependent manner. The histological lesions included congestion of cortical blood vessels, interstitial haemorrhage, interstitial fibrosis and tubular degeneration. Similar results were described by Zachariae et al. (1990) and Skretkowicz et al. (1996). Smeland et al. (1996) have reported that rats subjected to continuous infusion of 7-hydroxymethotrexate had yellow microscopic precipitations in their kidney tubules.

On the other hand, the simultaneous use of black seed oil – in the present study – resulted in an improvement of both the biochemical and morphological changes in the kidneys of methotrexate-treated animals. Similar results were obtained by El-Daly (1998) on cisplatin-induced toxicity in rats.

The present study did not reveal any abnormal histological changes between the brain of control animals and the brain of treated animals. Similar results were obtained by Moriuchi et al. (1996).

The results of the present study showed that there was a significant decrease – in a dose-dependent manner – of only the mean values of serum LH of methotrexate-treated animals, while the mean values of both testosterone and FSH were insignificantly decreased when compared with the control groups. Furthermore, histological sections of the testes of methotrexate-treated rats exhibited many morphological changes in also a dose-dependent manner, e.g. congestion of blood vessels, shrunken degenerated seminiferous tubules with increased interstitial tissue. Similar results were reported by Dethloff and Watkins (1992) in trimetrexate-treated rats. Dixon (1986) has speculated that virtually all stem cells would have to be destroyed to prevent repopulation of the germinal epithelium, but also suggested that a period as long as 12 cycles of the seminiferous epithelium (10–13 days for the rat) would be required for complete recovery from extensive drug – or chemical – induced degeneration and atrophy.

On the other hand, the simultaneous addition of black seed oil to animals treated by methotrexate resulted in an improvement of both the morphological architecture of the testes and the hypothalamic-pituitary-axis hormones. The mechanism of action of *Nigella sativa* oil could be explained on the basis

of the inhibitory effect of its active principle thymoquinone or its crude fixed oil on lipid peroxidation as previously reported by Houghton et al. (1995). They have further added that the crude fixed oil of *Nigella sativa* and pure thymoquinone inhibited the cyclooxygenase and 5-lipooxygenase pathways of arachidonic metabolism in the rat peritoneal leucocytes. Furthermore, both substances inhibited the non-enzymatic peroxidation in the ox brain phospholipid liposomes, but thymoquinone was about ten times more potent.

CONCLUSIONS AND RECOMMENDATIONS

Our results support the protective effect of black seed oil supplementation in reducing methotrexate side effects and toxicity related to the haematopoietic, gastrointestinal, hepatic, renal, and male reproductive systems. The data suggest that coadministration of black seed oil with methotrexate may have a potential clinical value, since black seed oil may alleviate the toxic effects of methotrexate in patients receiving this drug.

We recommended "of-course after further extensive research studies on humans" the addition of black seed oil to the inactive ingredients of the commercial tablet formula to reduce the side effects of the parent active ingredient methotrexate.

Future research work

Further studies on different dose schedules and time-intervals might be needed. Furthermore, more than one dose of black seed oil can be used in such studies to achieve the best results.

Further research work/s will also be needed to elucidate the exact mechanism/s by which *Nigella sativa* (L.) oil protects against methotrexate-induced side effects and toxicity.

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Zmírnění toxicity vyvolané methotrexatem u potkaních samců-albínů pomocí černuchy seté (*Nigella sativa* L.): biochemická, hematologická a histologická studie.

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V literatuře existují důkazy o vztahu mezi dávkou a reakcí na adjuvantní chemoterapii, i když publikované výsledky jsou kontroverzní. Využití vysokých dávek v režimu chemoterapie je omezeno sílou jejich toxicity. Cílem práce bylo hodnocení tolerance a účinnosti kombinační terapie s methotrexatem / olejem z černých semen ve srovnání s monoterapií methotrexatem u potkaních samců-albínů.

Methotrexat byl podáván potkaním samcům-albínům v nepřerušovaném režimu léčby, jenž by se mohl přirovnat k navrhované humánní léčbě. Zvířatům byl lék podáván orálně s methotrexatem s olejem z černých semen nebo bez něho po dobu pěti následujících dní a pak následovalo sedmidenní období rekonvalescence. Cyklus dávkování a zotavování se opakoval během čtyř cyklů. Změny v tělesné hmotnosti, hepatické, renální a testikulární funkce a změny v hematopoézním systému (erytrocyty, leukocyty v „celkovém a diferenčním počtu", krevní destičky a hemoglobin) a hepatická, gastro-intestinální, testikulární a mozková morfologie byly použity jako ukazatele hodnocení toxicity methotrexatu.

Léčba pomocí samotného methotrexatu měla za následek: a) značný pokles tělesné hmotnosti; b) značný pokles hepatické, renální a testikulární funkce; c) značný pokles hematopoézních ukazatelů; d) značnou proměnlivost v morfologických změnách ledvin, testes a střeva. Naproti tomu průvodní léčba methotrexatem a olejem z černých semen vyvolala reverzibilitu poklesu tělesné hmotnosti způsobenou methotrexatem, jakož i funkcí orgánů a morfologie. Výsledky naznačují, že olej z černých semen je farmakologickou protilátkou při toxicitě vyvolané methotrexatem, a proto v kombi-

naci s methotrexatem může sloužit jako základ, kterým lze dosáhnout intenzivnější a účinnější terapie methotrexatem.

methotrexat; olej z černých semen; biochemie; hematologie; histologie; potkani-albíni

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