International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(1): 602-605

Received: 15-11-2017
Accepted: 17-12-2017

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Hematological alterations in methotrexate induced toxicity in rats and its amelioration by *Tinospora cordifolia*

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Abstract
Methotrexate (MTX), an anti-neoplastic drug and is also used against psoriasis and rheumatoid arthritis. The present study was carried out at Department of Pathology, Veterinary College, Bangalore, India from July 2013 to December 2013 to assess the hematological changes following MTX administration in Wistar albino rats and its amelioration by extract of *Tinospora cordifolia* (TC) for a period of 45 days. Rats were divided into five groups comprising of control and treatment groups. The dosage used for MTX and TC extract was 5mg/kg body weight intraperitoneally and 200mg/kg by oral gavaging, respectively. The MTX treated rats showed significant decrease in haemoglobin, TEC, TLC and platelet count as compared to normal control animals. Supplementation of TC to MTX treated rats significantly improved the hematological parameters. The study concluded that damage to the hemopoitic system evident by deranged hematological parameters can be prevented by administration of extract of *Tinospora cordifolia.*

Keywords: Methotrexate, hematological changes, *Tinosporacordifolia*

Introduction
Methotrexate (MTX) is a chemotherapeutic agent widely used in the treatment of several types of cancers and various inflammatory diseases such as rheumatoid arthritis and psoriasis [1, 2]. It is an antimetabolite and an analogue of folic acid. It has been shown that MTX toxicity has severe side-effects on the hematopoietic system apart from other toxicities. MTX is known to cause highly significant reduction in RBC, haemoglobin, packed cell volume (PCV), white blood cells (WBC), neutrophil and lymphocytes of rats [3].
The adverse effects caused by the continuous or intermittent use of MTX are well known. Though folic acid is being prescribed routinely along with MTX, it has failed to reduce the adverse effects caused by MTX. Many drugs are continuously being tried to prevent/reduce the adverse effects of MTX [4].
Tinospora cordifolia (TC) is an important drug of Indian Systems of Medicine (ISM) and used in medicines since times immemorial. The drug is well known Indian bitter and is prescribed in fevers, diabetes, dyspepsia, jaundice, urinary problems, skin diseases and chronic diarrhoea and dysentery. It has been also indicated useful in the treatment of heart diseases, leprosy, helmenthiasis and rheumatoid arthritis. TC is known to contain various chemical constituents belonging to different classes such as alkaloids, diterpenoid lactones, glycosides and steroids and the most important biological properties are antioxidant, anti-diabetic, anti-inflammatory, anti-arthritic, anti-stress, hepatoprotective, immunomodulatory and anti-neoplastic properties [5]. The stem of the plant can be used by traditional practitioners for various therapeutic purposes like for treatment of jaundice, emaciation, skin ailments, diabetes, anemia, dyspepsia, chronic diarrhoea, dysentery, urinary problems and various infectious diseases [6-8].
Considering the above beneficial properties of TC, the present study was undertaken to evaluate the efficacy of *Tinospora cordifolia* in ameliorating the toxic effects methotrexate on the haematopoetic system in Wistar albino rats.

Materials and Methods
Methotrexate (Folitrax-15), used for induction of hepatotoxicity in rats was procured from IPCA Laboratories ltd, Mumbai, India.

Tinospora cordifolia aqueous stem-extract was obtained from Kisalaya Herbals ltd, Indore Madhya Pradesh, India. The extract was weighed and dissolved in water to make the required final concentration for administration to the individual experimental animals.

Healthy adult Wistar albino rats of both sex and weighing approximately, 140-150 grams procured from an approved laboratory animal supplier were used for the study. The animals were maintained under standard laboratory conditions and fed with *ad libitum* standard commercial rat feed and clean drinking water. The duration of experiment was of 45 days and prior permission was obtained from the Institutional Animal Ethics Committee (IAEC) for the conduct of the experiment.

Experimental design: The rats were maintained under standard laboratory conditions for a period of 15 days for acclimatization in the experimental animal house. The rats were divided, based on the body weight, into five groups with twelve rats in each group.

Group I: Negative control - injected with 0.5ml sterile PBS intraperitoneally on day 1 and gavaged with PBS daily.

Group II: Positive control- hepatotoxicity induced with administration of methotrexate at 5mg/kg body weight intraperitoneally for three consecutive days

Group III: Supplemented with *Tinospora cordifolia* extract at the dose rate of 200 mg/kg body weight concurrently with administration of MTX.

Group IV: Supplemented with *Tinospora cordifolia* extract at the dose rate of 200mg/kg body weight 10 days prior to induction of hepatotoxicity by MTX.

Group V*: Tinospora cordifolia* administered control animals supplemented with aqueous extract of *Tinospora cordifolia* alone at the dose rate of 200mg/kg body weight.

Experimental induction of toxicity in rats

To induce toxicity, the rats were fasted overnight and injected with methotrexateat5 mg/kg body weight by intraperitonial route for three successive days. The negative control animals were injected intraperitoneally with PBS alone. The rats in Group III were supplemented with extract of *Tinospora cordifolia* at 200 mg/kg body weight, intraperitoneally for 45 days along with methotrexate at the rate of 5 mg/kg body weight by intraperitonial route for three successive days. The rats in Group IV were supplemented with extract of *Tinospora cordifolia* at 200 mg/kg body weight, by oral gavaging, 10 days prior to and for 45 days following induction of toxicity with methotrexate

Hematological Parameters

On the scheduled days of blood collection (7^{th}, 14^{th}, $28^{th}$and 45^{th} day of the experiment), few drops of blood was collected from the retro-orbital flexus, in a vial containing ethylene diamine tetra acetic acid (EDTA) as an anticoagulant for evaluation of hematological parameters such as total erythrocyte count (TEC), total leukocyte count (TLC), total platelet count and hemoglobin (Hb). For the hematological evaluation, the auto hematology analyzer (BC-2800 VET, Mindray), was used.

Statistical analysis: Statistical analysis was performed using the statistical software Graph Pad Prism, version 6.0 for windows. Mean values and standard error were calculated and all values were expressed as Mean (± SE). The data were analyzed by two-way analysis of variance (ANOVA).

Results

The effect of MTX administration on the hematological parameters and various serum enzymes was analyzed. The results indicated that MTX caused a significant decrease ($P<0.01$) in the levels of hemoglobin, TEC, TLC and platelets (Table1, 2, 3, 4) in Group-II when compared to other groups, throughout the duration of the experiment.

Administration of aqueous extract of TC ameliorated the deleterious effects of MTX which was reflected by significant improvement ($P<0.01$) in the levels of hemoglobin, TEC, TLC and platelets in the animals of Group-III and Group-IV (Table1, 2, 3,4) and treatment with TC significantly ameliorated the deleterious effects of MTX.

Table I: Mean Values of Haemoglobin (g%) of Different Groups at Different Time Intervals

Groups	Days post treatment			
	7	14	28	45
Group I (NC)	12.65 ± 0.56^{a}	12.78 ± 0.64^{a}	12.78 ± 0.78^{a}	13.41 ± 0.68^{a}
Group II (MTX)	10.36 ± 0.25^{b}	10.40 ± 0.24^{b}	10.46 ± 0.69^{b}	12.10 ± 0.68^{b}
Group III (MTX+TC)	10.15 ± 0.57^{bc}	10.37 ± 0.57^{bc}	13.68 ± 0.22^{ac}	14.61 ± 0.65^{ac}
Group IV (TC+MTX)	13.96 ± 0.45^{ad}	14.68 ± 0.34^{ad}	14.61 ± 0.39^{ad}	14.67 ± 0.43^{ad}
Group V (TC)	15.22 ± 0.41^{ed}	15.06 ± 0.41^{ed}	15.31 ± 0.55^{ecd}	15.83 ± 0.36^{ecd}

Values with different superscripts in a column vary significantly at $p<0.01$

Table II: Mean Values of TEC ($X10^{6}$ /Mm^{3}) of Different Groups at Different Time Intervals

Groups	Days post treatment			
	7	14	28	45
Group I (NC)	7.84 ± 0.24^{a}	8.05 ± 0.20^{a}	7.66 ± 0.41^{a}	7.05 ± 0.43^{a}
Group II (MTX)	6.24 ± 0.15^{b}	6.37 ± 0.20^{b}	6.91 ± 0.42^{b}	7.34 ± 0.30^{a}
Group III (MTX+TC)	7.06 ± 0.59^{ab}	7.30 ± 0.58^{ab}	8.35 ± 0.23^{ac}	8.95 ± 0.16^{c}
Group IV (TC+MTX)	7.95 ± 0.29^{a}	8.25 ± 0.40^{a}	8.65 ± 0.36^{ad}	8.95 ± 0.16^{c}
Group V (TC)	9.53 ± 0.29^{e}	9.60 ± 0.25^{e}	9.27 ± 0.15^{cde}	9.05 ± 0.20^{ce}

Values with different superscripts in a column vary significantly at $p<0.01$

Table III: Mean Values of TLC ($X10^3$ /Mm^3) of Different Groups at Different Time Intervals.

Groups	Days post treatment			
	7	14	28	45
Group I (NC)	7.38±0.12[a]	7.45±0.19[a]	7.59±0.29[a]	7.59±0.13[a]
Group II (MTX)	5.75±0.21[b]	5.65±0.19[b]	6.15±0.22[b]	6.95±0.36 [a]
Group III (MTX+TC)	6.00±0.18 [bc]	6.03±0.28 [bc]	7.49±0.25 [ac]	8.62±0.28 [ac]
Group IV (TC+MTX)	7.64±0.24 [ad]	7.92±0.38 [ad]	9.04±0.28[d]	8.97±0.21 [cd]
Group V (TC)	7.70±0.21 [ae]	8.64±0.26 [de]	9.05±0.39 [de]	9.37±0.47[cde]

Values with different superscripts vary significantly at $p<0.01$

Table IV: Mean Values of Platelets ($x10^3$ /mm^3) of Different Groups at Different Time Intervals

Groups	Days post treatment			
	7	14	28	45
Group I (NC)	320.84±13.05[a]	327.17±13.35[a]	343.00±18.32[a]	337.34±16.29[a]
Group II (MTX)	204.50±17.81[b]	193.34±16.99[b]	244.17±18.58[b]	226.00±22.96[b]
Group III (MTX+TC)	251.67±15.95[ab]	257.00±14.63[ab]	295.17±22.71[ab]	299.34±19.14[ab]
Group IV (TC+MTX)	297.00±17.88[a]	292.67±32.59[a]	342.84±17.65[a]	350.00±24.36[a]
Group V (TC)	390.50±25.97[e]	382.84±20.98[e]	391.50±15.18[e]	401.67±21.94[e]

Values with different superscripts vary significantly at $p<0.01$

Discussion

The present study revealed significant hemoglobinemia, erythrocytopenia, leucopenia and thrombocytopenia in MTX treated rats throughout the experimental period indicate the direct toxic effect of methotrexate on the blood cells and hematopoietic system. Similar observations in blood parameters following MTX administration has also been reported earlier [9, 10, 2].

Methotrexate, being a cytotoxic drug, is reported to cause acute renal failure, bone marrow suppression and hepatotoxicity.MTX interferes with DNA synthesis, repair and cellular replication and affects mainly actively proliferating cells such as bone marrow cells, intestinal mucosal cells and urinary bladder cells. Bone marrow suppression due to MTX has been reported in several clinical trials with MTX for neoplastic conditions, rheumatoid arthritis and psoriasis cases in humans [11]. However, bone marrow toxicity with MTX is a late complication of treatment [12, 13]. In the present study, reduction in the values of blood parameters could be attributed to the mild to moderate suppression of bone marrow by MTX. In addition, profuse bleeding due to intestinal injury by MTX and free radical induced red cell damage could have contributed for lowered erythrocyte count and hemoglobin values.

In co-treatment group (MTX + TC), the hematological parameters such as hemoglobin, total erythrocyte count, total leukocyte count and total platelet count showed marked improvement from day 28 of experiment in comparison to MTX treated group. However, pretreatment with TC prior to MTX administration showed improvement in all the studied hematological parameters from day 7 of the experiment. In addition, when the values were compared between the groups III and IV, the TC pretreated group (IV) showed significant improvement. Similar protective effects of TC and improvement of haematopoetic function following induced toxicity in mice has also been reported earlier [14, 15, 5].

Tinospora cordifolia Meirs (Menispermiaceae) commonly known as Guduchi (Sanskrit) and Giloy (Hindi); is a glaborous, climbing, succulent herb commonly found in hedges and is a native of India and thrives easily in tropical regions. This plant is widely used in Ayurveda as vitaliser, antidiabetic, hepatoprotective, antipyretic, antistress, antiulcer and immunomodulatory agent. It was therefore considered that antioxidant properties of *T. cordifolia* may be substantially responsible for these medicinal effects.

The extract of plant, *Tinospora cordifolia* may have protection through various mechanisms such as free radical scavenging, calcium channel blocking, inhibition of lipid peroxidation, metal chelation capabilities, immunomodulation, ennhancement of DNA repair, hepatoprotective and stimulation of haematopoetic cellular proliferation and differentiation [16]. Several reports have suggested that *T. cordifolia* is used in the treatment of multiple disorders; it also enriches the blood [17, 18, 8]. In the present study, T. cordifolia may plausible be acting in a competitive and comprehensive mode to amelioarate the MTX induced oxidative stress and suppression of haematopoetic activity in rats.

Extrapolation of the data from the earlier reports *in-vitro* experiments as well as *in-vivo* studies and the present study warrants further studies employing the broader perspectives to determine improvement in dosage and duration of treatment with TC, so as to define the maximum effective dose. The results might possibly be of a better magnitude, if the active principle of the test compound is used for the study instead of the crude extract.

Conclusion

The study highlighted the beneficial effects of *Tinospora cordifolia* at 200 mg/kg body weight in ameliorating the toxic effect on MTX induced hematological changes in Wistar albino rats and the possible preventive role of TC against MTX induced toxicity.

Acknowledgement

Authors are the thankful to the Vice Chancellor, KVAFSU, Bidar and Dean, Veterinary College, Bangalore for providing the necessary facilities.

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