The Pharma Innovation Journal 2021; 10(7): 1434-1436

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(7): 1434-1436

www.thepharmajournal.com
Received: 17-05-2021
Accepted: 28-06-2021

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Study of ameliorative effect of quercetin on methotrexate induced toxicity on body weights and haematological parameters in albino Wistar rats

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Abstract
The amelioration effect of quercetin on Methotrexate induced toxic effect on body weights and haematological parameters were studied in *Wistar* rats. The experiment was carried on 48 healthy albino *Wistar* rats (200-220 g) according to the guidelines and prior approval of Animal Ethics Committee. Animals were divided into four groups consisting of 12 in each group. Group1-Control, group 2-Methotrexate treated rats @ 8mg/kg b.wt (i.p) weekly once. Group 3-treated with Methotrexate @ 8mg/kg b.wt, (i.p) weekly once and quercetin @ 80mg/kg b.wt, Orally daily. Group 4-treated with quercetin @ 80mg/kg b.wt, orally daily. The experiment was carried out for a period of 28 days and rats were sacrificed on 14th and 28th day of experimental study. Significant (P< 0.05) reduction in b. wts were recorded in group 2 rats. All the Heamatological parameters were significantly reduced in group 2 and significant improvement of all parameters was noticed in ameliorative group (group 3).

Keywords: Methotrexate, Quercetin, body weight, haematology, albino *Wistar* rats

Introduction
Methotrexate (MTX) is a WHO 'essential medicine' that is now widely employed as a first-line treatment in auto-immune, inflammatory diseases such as rheumatoid arthritis (RA), psoriasis and Crone's disease (Bedoui *et al*., 2019) [4]. Both the methotrexate and the methotrexate polyglutamate inhibit the enzyme dihydrofolate, which catalyse the conversion of Dihydrofolate into tetrahydrofolate, the active form of folic acid (Mikhaylov *et al*., 2019) [12]. Tetrahydrofolate is necessary for the synthesis of the nucleotides of both DNA and RNA. Methotrexate-polyglutamate further inhibits the de novo purine synthesis of both purine and thymidylate synthase, thereby inhibiting DNA synthesis. Methotrexate is known for its toxic ef fects involving most of the rapidly multiplying cells of the organs which include bone mar-row, liver, lungs, kidneys, gut and gonads (Patel *et al*., 2014) [14]. MTX has direct toxic effect on blood cells and haemopoitic system and indirectly could be due to reduced feed intake thereby leading to hypoproteinemia. Deepak *et al.* (2018) [6].
Quercetin (QE), belonging to a subclass of flavonoids called flavanols, is a frequently studied dietary flavonoid ubiquitously present in various vegetables, fruits, seeds, nuts, tea and red wine (Formica and Regelson, 1995) [8]. It has the ability to prevent the oxidation of low-density lipoproteins by scavenging free radicals and chelating transition metal ions (Bentz, 2009) [5], thereby aiding in the prevention of diseases, such as cancer, atherosclerosis, and chronic inflammation (Murota and Terao 2003) [13].
Based on the above facts, the present experiment was designed to study the protective effect of QE against MTX induced haematological alterations.

Materials and Methods
Experimental Animals
Rattus norvegicus (*Wistar* strain) rats (48) weighing 200-220g, bred at M/S Vyas Labs (CPCSEA registered No.17/22/C.V.Sc, Hyd/IAEC1), Medchal, Malkajgiri were used for this research. The experiment was carried out according to the guidelines and prior approval of Institutional Animal Ethics Committee (IAEC-No.5/23/C.V. Sc., Hyd).

Chemical source

Methotrexate was obtained from Nawab Mehdi Nawaj Jung (MNJ Govt Cancer Hospital) Redhills Road, Lakadikapul, Hyderabad, Telangana and Quercetin (QE) obtained from Healthvit, West Coast Pharmaceutical Works Ltd, Ahmedabad, India.

Experimental design

Table 1: Show the group of Treatment

Groups	No. of rats	Treatment
Group 1	12	Control
Group 2	12	Methotrexate at the rate of 8mg/kg b.wt weekly once, intraperitoneally.
Group 3	12	Methotrexate at the rate of 8 mg/kg b.wt weekly once, intraperitoneally + Quercetin @ 80 mg/kg b.wt, orally.
Group 4	12	Quercetin at the rate of 80 mg/kg b.wt, orally.

Body weight (B.wt) gain (s)

Individual animal body weights of all the rats were recorded by using electronic balance on day one (initial b.wt on 0th day) soon after arrival and subsequently on 7th, 14th,21st and 28th day of experiment to study.

Haematology

Six rats from each group were sacrificed on 14th day of experiment and remaining were sacrificed on 28th day. Prior to blood collection, the experimental rats were put to fast for12 hours. Just before sacrifice, the rats were anesthetized and 1mL of blood was collected from retro-orbital plexus with the help of capillary tube (3 mm) in an anticoagulant (K_3- EDTA) coated vacutainers {13mm x 75mm, 4 mL (Rapid Diagnostics Pvt, Ltd., Delhi)} to carry out all haematological parameters *viz.* TEC, Hb concentration, PCV and TLC by using an automated whole blood analyzer (Huma count, med source Ozone Biomedical Faridabad, Haryana) and results were tabulated for statistical analysis.

Statistical analysis

Data obtained were subjected to statistical analysis by applying one-way ANOVA using statistical package for social sciences (SPSS) version 16.0. Differences between the means were tested by using Duncan's multiple comparison tests and significance level was set at $P< 0.05$ (Snedecor and Cochran, 1994) [17].

Results and Discussion

MTX effect on B.Wts

The present study revealed a significant ($P< 0.05$) higher mean values of weekly b.wts were recorded in control group and significant lower mean values were observed in group 2 rats on 7th, 14th, 21st and 28th day respectively. The adverse affect on body weight might be due to reduced feed and water intake and decreased basal metabolism due to toxic metabolites of MTX, increased stressogenic activity and diarrhea. These findings were in accordance with the reports of Arisha *et al.* (2017) [2] and Khafaga *et al.* (2018) [10]. The rats in group 3 showed significant improvement in body weight as compared to group 2 indicating QE might have reversed the supress effect of methotrexate on body weight. Haleagrahara *et al.* (2018) [9] also observed similar effect on body weights when treated with quercetin (Table 2)

MTX effect on haematological parameters

A significant decrease in total erythrocyte count, haemaglobin concentration and packed cell volume and TLC was noticed in group 2 rats when compared with group 1 on 14th and 28th day of experimental period. These adverse effects might be due to direct toxic effect of methotrexate on blood cells and haemopoitic system and indirectly could be due reduced feed intake there by leading to Hypoproteinemia. These findings were in accordance with Deepak *et al.* (2018) [6] and Mhatre *et al.* (2016) [11]. A significant restoration of PCV and TEC was noticed in group 3 compared with group2 on 14th and 28th day of experiment. QE natural food-derived antioxidants presuming to have haemo protective effect as similar with other photochemical like withaferin (Vellanki *et al.* 2019 and priyanka *et al.* 2020) [18, 15], and pomegranate juice (ellagic tannins, ellagic acid and gallic acid) (Shaik *et al.* 2021) [16]. Among flavonoids, quercetin (QE) is considered an excellent free-radical scavenging antioxidant, even if such an activity strongly depends on the intracellular availability of reduced glutathione. QE might have interfered with MTX induced toxic action on membrane lipoproteins and direct heamolysis of erythrocytes (Asgary *et al.*, 2005).[3] QE is also a superior compound to inhibit both oxidative damage and destruction of premature erythrocytes due to presence of 3-OH and greater number of hydroxyl groups in its chemical structure (Afanas Tev *et al.* (2001) [1].

There was a significant improvement in TLC in group 3 when compared with group 2. This might be due to protection of erythrocytes and restoration of hematopoitic system. Similar protective effects of quercetin and improvement of TLC values following MTX induced toxicity has been reported earlier (Donmez *et al.*, 2019) [7]. (Table 3)

Table 2: Body weights (g) at weekly intervals in different groups

Group	Day 7	Day 14	Day 21	Day 28
1 (Control)	222.53±0.23^{a}	224.21±0.22^{a}	226.25±0.29^{a}	222.40±0.28^{a}
2 (Methotrexate)	206.70±0.67^{c}	187.35±1.39^{c}	167.83±1.04^{c}	153.20±1.63^{c}
3 (MTX + QE)	217.1±0.31^{b}	210.93±1.43^{b}	201.06±2.18^{b}	190.30±6.55^{b}
4 (Quercetin)	221.62±0.31^{a}	221.50±0.54^{a}	226.03±0.95^{a}	229.80±0.57^{a}

Values are Mean ± SE (n=6); One-way ANOVA

Means with different superscripts in a column differ significantly at ($P< 0.05$)

Table 3: Haematological Parameters in different groups

Group/Day	Group 1		Group 2		Group 3		Group 4	
	Day 14	Day 28	Day 14	Day 28	Day 14	Day 28	Day 14	Day 28
Haemoglobin (g %)	15.85±0.06^{a}	16.40±0.08^{a}	12.54±0.11^{c}	10.03±0.10^{c}	14.14±0.12^{b}	10.71±0.21^{b}	15.79±0.11^{a}	16.06±0.19^{a}
TEC (Mill/µL)	8.78±0.17^{a}	9.48±0.03^{a}	6.98±0.11^{c}	5.54±0.15^{c}	8.16±0.20^{b}	9.02±0.06^{b}	8.91±0.08^{a}	9.19±0.09^{ab}
PCV (%)	54.46±0.20^{a}	57.77±0.31^{a}	44.33±0.66^{c}	33.89±0.55^{c}	49.65±0.30^{b}	53.61±0.34^{b}	53.52±0.53^{a}	57.16±0.60^{a}
TLC (Thousand/µL)	17.68±0.86^{a}	24.80±0.52^{a}	6.04±0.02^{b}	5.22±0.12^{c}	7.70±0.18^{b}	10.87±0.09^{b}	19.66±1.18^{a}	25.56±0.37^{a}

Values are Mean ± SE (n=6); One-way ANOVA

Means with different superscripts in a column differ significantly at (P< 0.05)

Conclusion

MTX exposure at the rate of 8 mg/kg b.wt (i.p) leads to significant reduction in body weights and marked reduction of haematological parameters which might to due to toxic effect of MTX on GIT tract and haemopoitic system and Quercetin as a potent anti-inflammatory and anti-oxidant capable of attenuating these toxic effects of MTX.

Acknowledgement

Authors are highly grateful to PV Narsimharao Telangana Veterinary University and NAHEB, Hyderabad for providing facilities to carryout this smooth clinical research.

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