**The Possible Protective Effect Of Quercetin On Methotrexate Induced Hepatotoxicity In Adult Albino Rats: A histological, Biochemical And Immunohistochemical Study.**

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## Abstract

**Background:Methotrexate(Mtx) is an antineoplastic and immunosuppressive drug. That may cause hepatotoxicity, whereas quarcetin has anti-inflammatory and antiproliferative properties.**

**Aim of the work: This study aimed to investigate the possible protective and therapeutic effects of quercetin against methotrexate (Mtx)-induced hepatotoxicity with biochemical and histopathological studies in rats**

**Materials and methods: twenty six Wister albino rats were equally divided into four experimental groups: Control (group I), Mtx group (group II) rats received (single dose of Mtx 20 mg/kg i.p.). QCT protective group (group III) rats pre-treated with QCT (20 mg/kg orally daily for 7 days then Mtx administration. QCT therapeutic group (group IV) rats cotreated with Mtx(20 mg/kg for i.p, single dose)then QCT were given as before .Following treatment, the animals were sacrificed, and liver tissue samples were histopathologically evaluated using H&E, Masson's, PCNA staining, and serum transaminases were measured and statistically compared across all groups**

**Results:Group II (Mtx group) demonstrated hydrobic degeneration of hepatocytes, congestion of hepatic sinusoids ,central veins and portal veins .An apparent increase in collagenous fibers distribution around the central vein and portal tract was detected .In groups III (protected QCT), and group IV (QCT therapeutic groups), showed less histological injury compared to Mtx group as regards liver sections ,but pretreatment with QCT in group III was more effective than in group IV except for mild dilation of sinusoids**

**Conclusion: Methotrexate has a deleterious effect on the liver. Quaricetin may be a potential adjuvant drug to reduce the hepatic side effects observed during Mtx therapy for various clinical conditions.**

 **Key words:** Rats ,Quercetin; Methotrexate; side effect; Liver.

**Introduction**

Cancer is characterized by abnormal growth and proliferation of cells. Variable techniques are used for the treatment of cancer including chemotherapy, radiation and surgery. Chemotherapy is the most common method of treatment [1]

One of the essential therapies for tumors is the chemotherapy and many anti-cancer drugs have been produced . Although, the systemic toxicity and side effects results from these therapies restricts their wide spread uses [2,3]

It is given systemically to patients to control the cancerous cells proliferation but chemotherapy unfortunately has no selective action and cannot differentiate between cancer cells and healthy cells[4]

Methotrexate (Mtx), a folic acid analogue drug , and has been widely applied chemotherapeutic agent for treatment of several malignancies, so it is considered an effective cytotoxic agent. It is known that the application of chemotherapeutics causes acute toxic effects in multi-organ systems. In addition, methotrexate is a potent substance as anti-inflammatory and immunosuppressive therapies and prescribed for healing of many chronic inflammatory disorders such as rheumatoid arthritis, psoriasis, Crohn's disease[5,6]

Mtx is considered the first option for the treatment of most cases due to the well-experienced treatment option and the comparable low price . Though, hepatotoxicity is the mainly severe side effect of Mtx and high over doses of Mtx may lead to stellate cell hypertrophy, hepatic fibrosis steatosis and an isonucleosis [7]

Natural products particularly possessing antioxidants properties have been considered as possible nutraceuticals to increase the effectiveness of chemotherapeutic agents and restrict their adverse effects[8]*.*Quercetin (3,3′, 4′, 5,7-pentahydroxyflavone; QR) polyphenolic compounds found with sufficient quantities in food sources and plants. It is mainly found in fruits,vegetables, tea, other aromatic plants and red wine [9]*.*QCT was considered a therapeutic material to eliminate different toxicities, particularly, renal toxicity [10] heart toxicity [11]*,*Nervous system toxicity[12] and liver toxicity[13]. Additionally, QCT has been proved to have antioxidant[14],antimicrobial[15],anti-inflammatory[16]and anticancer activities[17] . The main reason for antioxidant activity of QCT may be attributed to the high diffusion of QCT into cell membranes to sweep oxyradicals [18]

**The present study aimed** to study the useful influences of QCT on Mtx-induced hepatotoxicity

## Materials and methods

## Animals and experimental design

**Chemicals**

Quercetin (3,3′,4′,5,7-pentahydroxy flavanone, L21600) was purchased from Enzo Life Sciences (Farmingdale, New York, USA) and Methotrexate (25 mg/ml ) was purchased from Hospira (UK) .

**Animals**

A total of 24 adult male Sprague–Dawley rats, their ages about 8 weeks and weighing 200–250 gm were used in this study. The rats were raised in Animal House, Faculty of [Veterinar](https://www.google.com/search?client=firefox-b-d&q=veterinary&spell=1&sa=X&ved=0ahUKEwjk4OyA2JDhAhVvxoUKHUrTB4QQkeECCCgoAA)y Medicine, Mushtohar , Benha University. The design of the experiment was approved according to Animal Ethics Committee. Animals were fed standard diet and water *ad libitum* and under controlled temperature and light (24°C; 12 h light/12 h dark cycles, respectively). The rats were divided randomly into 4 experimental groups(6rats/group), as follows:

1. **Control group (I)**: Received distilled water single dose equivalent to Mtxby intraperitoneal injection
2. **Mtx-treated group (II):**Animals received Mtx(single dose 20 mg/kg i.p.) according to [19]
3. **QCT protected group (III):**Animals received quercetin(20 mg/kg) orally daily for 7 days[20] before Mtx administration
4. **QCT therapeutic group (IV):** Animals received orally over 7 days 20 mg/kg b.w. QCT daily after Mtx administration.

At the end of the experimental period, the rats were decapitated after anaesthetized with 10 mg/kg Xylazine and 50 mg/kg Ketalar, i.p. , blood samples were collected for chemical analysis , whereas the liver tissues were dissected and were fixed in 10% neutral formalin for histopathological examination.

**Histopathological analysis**

Fixed materials were dehydrated in ascending concentrations of ethyl alcohol and embedded in paraffin wax and of 5-micrometer thick sections were prepared and subjected to staining with hematoxylin-eosin (Hx&E) , and Masson’sTrichrome (MT) stains.

**Immunohistochemical staining of PCNA:**

Samples of rat liver embedded in Paraffin were deparaffinized and hydrated. The sections were incubated with 3% hydrogen peroxide for 5 minutes to stop endogenous peroxidase activity. Then the sections were incubated with PCNA monoclonal antibody over night and rinsed for five minutes with phosphate buffer saline (PBS). The monoclonal antibody was then linked with biotinylated goat anti-mouse IgG antibody for thirty minutes. The sections samples were incubated with streptavidin-conjugated peroxidase for 35 minutes, after being washed with PBS for 3-5 minutes. Development of a brown colored reaction was observed after adding of 3, 3-diaminobenzidine tetrahydrochloride solution(DAB) for 5 minutes and washed after that in distilled water. Samples were countered-stained with hematoxylin **[21]**. PCNA positive cells were estimated in 10 randomly chosen, non-overlapping fields and were expressed as the number of PCNA positive cells/mm2.

**Biochemical analysis**

Blood samples were obtained from retro-orbital plexus of all rats, 1ml from the blood in tubes containing disodium EDTA. The blood samples were centrifuged at 3000 rpm for 20 min for separation of plasma which were immediately used for biochemical determinations . Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin(TB) and alkaline phosphatase (ALP) were determined by using commercial biochemical kits and REFLOTRON ‘PLUS machine by Roche, USA.

**Morphometric study:**

The average percentages area of both collagen deposition and PCNA immuno-expression of each rat were estimated in 5 images from five non-overlapping fields using Image-Pro Plus program version 6.0 (Media Cybernetics Inc., Bethesda, Maryland, USA).

**Statistical analysis**

All the obtained results from the study were tabulated and analyzed statistically using IBM SPSS Statistics software for Windows, Version 19(IBM Corp., Armonk, NY, USA). To match the variations between the groups One-way analysis of variance (ANOVA) with Post Hoc LSD test was used. The data were expressed as the mean (M) ±standard deviation (SD)in each test, and the differences were considered to be significant at P≤ 0.05 and non-significant at P>0.05.