ORIGINAL ARTICLE

Protective Effects of Turmeric (Curcuma Longa) Against Methotrexate Induced Liver Toxicity in The Albino Mice

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ABSTRACT

Objective: To study the protective effects of Turmeric against hepatic damage brought on by Methotrexate.

Study Design: Randomized controlled trial.

Place and Duration of Study: This experimental research was conducted at Animal House of Institute of Basic Medical Science (IBMS), Khyber Medical University, Peshawar from 10June 2017 to 25 June 2018.

Materials and Methods: A total of 28 adult albino mice were divided into four groups. Group A was control group. Control group received no medication. Group B received a daily dose of Turmeric extract (400 mg/kg) for 14 days. Group C received an intraperitoneally (I.P) injection of Methotrexate (40 mg/kg) on day 7. Group D received oral administration of Turmeric extract (400mg/kg) for 14 days and injection Methotrexate (40mg/kg) was administered intraperitoneally (I.P) on day 7. All mice were sacrificed on day 14. Weight of liver of all the animal was recorded. For data analysis Statistical package for social sciences (SPSS) version 21 was used. Quantitative variables were expressed as mean ±standard deviation and the significant difference was assessed using ANOVA test. The Chi square test was used to determine statistical significance based on the categorical variables. P value <.05 was significant statistically.

Results: On microscopic examination of liver tissue, abundant inflammatory cells were observed around the portal area in all mice (100%) of Methotrexate group (Group C) with increase in liver weight. In Methotrexate + Turmeric group (Group D) few inflammatory cells were observed with slight decrease in liver weight as compared to Group C in all mice (100%).

Conclusion: The results of this study revealed that Turmeric (*Curcuma Longa*) treatment protects liver tissue against Methotrexate-induced damage.

Key Words: *Curcuma Longa, Hepatotoxicity, Inflammatory cells, Methotrexate, Reactive oxygen species*

Introduction

Methotrexate is one of folic acid antagonists. It was initially used for acute leukemia in children. The effective use in treating other cancers followed thereafter.[1] Methotrexate has been commonly used in the treatment of a rheumatoid arthritis, psoriasis and other autoimmune disorders such as juvenile idiopathic arthritis for more than 40 years.[2] Methotrexate is effective in the treatment of systemic lupus erythematosus, vasculitis, inflammatory bowel syndrome and several other diseases of connective tissue.[3] It is also used for the treatment of leukemia and malignancies of different organs such as breast and lung.[4]

The most frequent side effects of Methotrexate are nausea, vomiting, loss of appetite, ulcers of mucosa, when taken in low doses.[5] After used as a continuous therapy, its other side effects are alopecia, low white cell count, increased risk of infection, GI bleeding, bone marrow suppression, hepatotoxicity and renal failure.[6,7,8]

Methotrexate and its metabolite-polyglutamated inhibit the dihydrofolate reductase enzyme, which converts dihydrofolate into tetrahydrofolate (The folic acid's active form). The nucleoside thymidine denovo can only be produced with folic acid. Methotrexate thus has an indirect effect on the thymidylate synthesis, which is required for synthesis of DNA[9]. Hepatocytes are damaged by

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reactive oxygen molecules such as hydrogen peroxide, hydroxyl radicals, and superoxide. Methotrexate inhibits NADP, which is needed by the glutathione reductase enzyme to neutralize reactive oxygen species.[10]

Herbs have a key part in the treatment of many liver illnesses because there are no effective pharmaceuticals that can protect the liver in allopathic medical procedures.[11] Numerous studies have demonstrated the significance of plant extracts in liver diseases .[12]

The use of Turmeric in Chinese and Indian medicine has a long history. [13] There are more than 300 ingredients in Turmeric, diarylheptanoids, monoterpenes, diterpenes, sesquiterpenes, phenyl propene, alkaloids, and curcuminoids are some of these ingredients.[14] It is used to treat inflammatory disorders, cancer, hepatitis and other hepatic disorders, Alzheimer's disease, skin diseases and rheumatoid arthritis[15].

The generation of reactive oxygen species (ROS), such as hydroxyl radicals and hydrogen peroxide, is inhibited by Turmeric. Turmeric's antioxidant properties thereby prevent the liver damage caused by free radicals.[16,17] Limited studies have been conducted to know the role of Turmeric in protection against Methotrexate hepatic damage. Therefore, this study was planned to–assess the defensive effects of Turmeric on Methotrexate induced hepatic toxicity in mice.

Materials and Methods

This randomized controlled study was conducted at the animal house of Institute of Basic Medical Sciences, KMU, and Peshawar from 10 June 2017 to 25 June 2018. Sampling technique was convenient sampling. Ethical clearance was taken from the ethical review board of Postgraduate Medical Department, Khyber Girls Medical College with reference number.3325/PGMED/KGMC. Male albino mice were included in study. Female albino mice were excluded. Adult male albino mice weighing 25 to 45 grams (5 to 7 weeks) were acclimated for two weeks in a 12-hour cycle of darkness and light at temperature of 22^{0}C. Mice were split between the control group (Group A) and the experimental groups B, C, and D. There were four mice in the control group (A) and eight mice each in groups B, C, and D. Group A received no medication. Group B was given Turmeric extract orally (400mg /kg) for 14 days. Group C received Injection Methotrexate (40mg/kg) intraperitoneally on day 7. Both Turmeric and Methotrexate given to Group D. Turmeric extract (400mg /kg) daily for 14 days was given to Group D. On day 7, injection of Methotrexate (40mg/kg) was administered intraperitoneally to Group D [17]. All mice were sacrificed on day 14. Weight of the livers of all the animal was recorded. Analytic balance was used to measure liver organ weight. The liver was preserved in formalin. By tissue processing, various liver sections were made. Eosin and hematoxylin were used to stain the slides. To observe the histological parameters, tissues were seen under a light microscope. Slides were examined by histopathologist. (Assistant Professor, MPhil histopathology).

For data analysis Statistical package for social sciences (SPSS) version 21 was used in this study. Quantitative variables were expressed as mean ±standard deviation and the significant difference was assessed using ANOVA test. The Chi square test was used to determine statistical significance based on the categorical variables and results. P value <.05 was significant statistically.

Results

The mean value of liver weight was significantly increased in Methotrexate Group i.e. 1.650 ± 0.17gm as compared to control group i.e. 1.352 ± 0.20gm. Whereas mean value of liver weight of mice treated with Methotrexate and Turmeric was significantly reduced i.e. 1.464 ± .016gm as compared to Group receiving Methotrexate ($p< 0.00$). The weight of liver of different groups is shown in figure 1.

On gross examination of mice liver in Methotrexate and Methotrexate + Turmeric group, there was no change in color, texture and consistency as compared to control group.

On histological examination of liver tissue, no periportal inflammation was present in all animals of control as well Group B (Turmeric). Abundant inflammatory cells were present around portal area in all eight animals receiving Methotrexate Group C (figure 2) with p value < 0.00 (table 1) which is statistically significant, where as in the Group D (Methotrexate plus Turmeric) few inflammatory cells were seen as compared to Group C with $p< 0.00$ (figure 3).

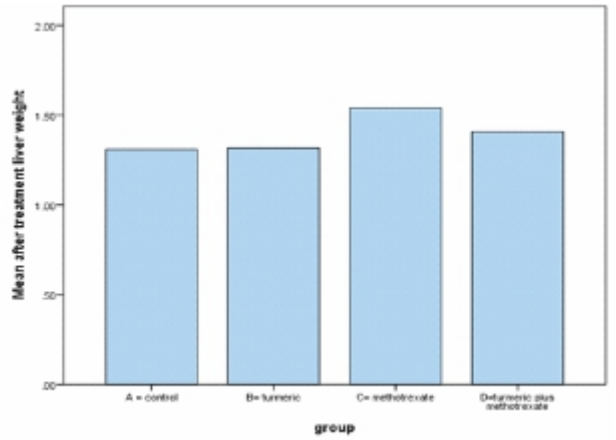

Figure 1: Increase in Weight of Liver of Methotrexate Group

Table I: The Effect of Methotrexate and Turmeric on Periportal Inflammation

Parameter	Control group A *n*=4	Turmeric group B *n*=8	Methotrexate group C *n*=8	Methotrexate plus Turmeric group D *n*=8	p value
Periportal inflammation	Absent n=4	Absent n=8	marked n=8	Mild n=8	0.00

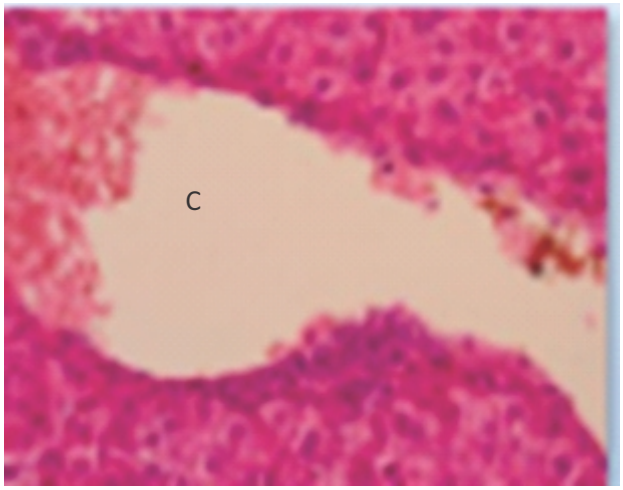

Figure 2: Photomicrograph of Liver Tissue of (MTX Group) Where " C" Shows Abundant Inflammatory Cells Around Portal Area At 40x

Discussion

Methotrexate has been highly criticized for its hepatotoxicity. If a medicine is used with a good ameliorative agent, the liver may be protected. Several researchers scientifically demonstrated that Curcuma longa (Turmeric) has anti-inflammatory, antimicrobial, anti-cancer and antioxidant properties[18,19]. Thus, Curcuma Longa (Turmeric) have capability to provide protection against oxidative stress induced by Methotrexate[20,21]

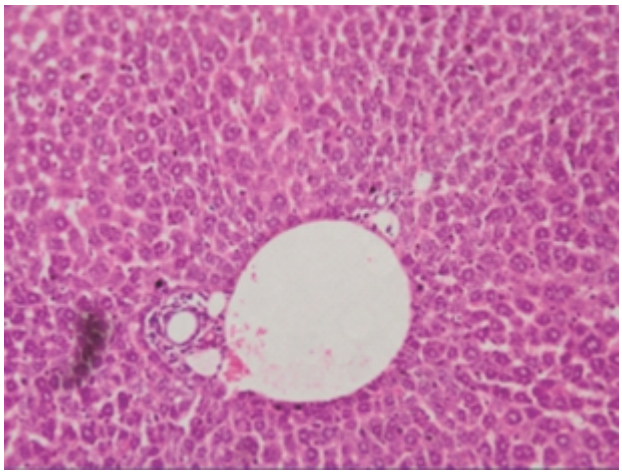

Figure 3: Photomicrograph of Liver Tissue of (MTX+ Turmeric Group) Where Few Inflammatory Cells Round Portal Area At 40x

Liver weight was significantly increased in Methotrexate group. Whereas in Methotrexate Turmeric group administration of Turmeric causes significant reduction in liver weight as compared to Methotrexate group. Liver weight increased because of diffuse inflammatory cellular infiltration, collagen deposition, mild hepatic edema and fatty infiltration.[22,23] Our findings concur with those made by Tag et al, who observed that administration of mulberry leaves extract causes significant reduction in liver weight of mice as compared to Methotrexate group.[24]

Significant periportal inflammation was observed in all eight animals in Methotrexate group. Few inflammatory cells are observed in Methotrexate Turmeric group. Our results correlate with the result of Adel Rezaei et al. In his study he also observed that periportal inflammation induced by Methotrexate is significant reduced by ethanolic extract of Turmeric (*Curcuma Longa*) [19]. The protecting effects of curcumin against liver damage were also observed by a study conducted by Erenoğlu etal.[25]

Conclusion

In conclusion, turmeric (*Curcuma Longa*) treatment protects liver tissue against Methotrexate-induced damage.

Recommendations

It is feasible to use Turmeric as a potential addition to a Methotrexate therapy regimen by conducting clinical trials to examine the herb's effects on human.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.

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