Original Article

Compare Oxidative Role of Beta-Carotene and Resveratrol (3 4 5 Hydroxystillbene) in Methotraxate Induced Hepatotoxicity on the Basis of Morphology and Catalase Activity

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ABSTRACT

Objective: Compare the oxidative role of β-carotene (BC) and Resveratrol (RSV) in Methotrexate (MTX) induced liver toxicity based on morphology and catalase activity.

Study Design: Experimental longitudinal study

Place and Duration of Study: This study was conducted at the Al-Tibri Medical College & Hospital from January 2021 to October 2021 for a period of 10 months.

Materials and Methods: 48 healthy Wistar albino rats were attained and then divided into 6 groups, Group I (Control Group) received normal saline, Group II was given BC, Group III was given RSV, Group IV was given MTX, Group V was given BC+MTX, and Group VI was given RSV+MTX for 24 days. On the 25th day the subjects were euthanized and liver sections for studying the nuclear diameter was fixed with formalin and stained using H&E staining. The nuclear diameter was measured with ocular counting scale on a light microscope. The liver homogenate was used to analyze catalase enzyme content. Data was analyzed using SPSS with all variables given in Mean± S.D. for statistical analysis paired-t test was done with the level of significance being P-value <0.05.

Results: Mean Nuclear Diameter of Hepatocytes were 5.067 ± 0.1256 in G-I, 5.233±0.0422 in G-II, 5.217±0.0601 in G-III, 5.733±0.0803 in G-IV, and 5.483±0.0307 in G-V, and 5.467±0.0494 in G-VI. Significant difference was seen when all the groups were compared with the MTX Group (G-I vs G-IV P=0.008, G-II vs G-IV P=0.002, G-III vs G-IV P=0.006, G-V vs G-IV P=0.048, & G-VI vs G-IV P=0.042). The level of CAT in each Group in (U/mg protein) were 143.5±3.21 in G-I, 143.15±2.87 in G-II, 149.17±3.85 in G-III, 92.71±1.93 in G-IV, 123.09±3.19 in G-V, and 125.5±1.95 in Group VI. Significant difference was seen when all the groups were compared with the MTX groups (G-I VS G-IV P=0.0001, G-II VS G-IV P=0.001, G-III VS G-IV P=0.001, G-V VS G-IV P=0.01, & G-VI VS G-IV P=0.01).

Conclusion: According to the study results both therapeutic agents were significantly effective in prevention of the toxic effects of methotrexate on liver. There potent antioxidant effect was evidenced through morphological restoration of liver architecture and catalase activity.

Key Words: methotrexate, liver, catalase

Citation of article: Ali SMM, Sughra S, Khan S, Ahsan I, Masood SH, Islam F. Compare Oxidative Role of Beta-Carotene and Resveratrol (3 4 5 Hydroxystillbene) in Methotraxate Induced Hepatotoxicity on the Basis of Morphology and Catalase Activity. Med Forum 2022;33(1):52-56.

INTRODUCTION

Liver is the biggest organ of the human body. Its weight is approximately 1500 Gm. This organ is a wedge shaped. It placed in right hypochondria and epigastrium and extending into left hypochondria. Naturally it is protected by rib cage and maintains its position by peritoneal reflection, followed by ligamentous attachment.[1, 2] Drug induced injury as a prolong time concern and diverting the attention towards it as a serious health issue and threatening situation for public. Moreover, it was came up as a main reason for an approved drug for the withdrawn from the market or Tag with the black box warning. The liver is the most vulnerable organ being damage by drugs, which further developed hepatotoxicity. Drug induced hepatotoxicity is unpredictable, and acute injury with various serious health problems. Hence it is established that the

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Received: November, 2021
Accepted: December, 2021
Printed: January, 2022

reactive metabolites formed from drugs are responsible for most cases of hepatotoxicity. Which are accompanied by the two reactive small molecules Reactive oxygen species (ROS) and Reactive nitrogen species (RNS).These are biomarkers to predict the drug induced hepatotoxicity.[3] One of the drug which creates hepatotoxicity is methotrexate. Methotrexate (MTX) is broadly used as second line of immunomodulation.[4] It is an antimetabolite and antifolate drug. The indication of methotrexate is in various diseases. It is used as a therapy of leukemia, lymphoma and in many solid tumors. It indicates in immune modulatory effects against inflammatory disease like psoriasis, inflammatory bowel disease and inflammatory arthritis. It is effective with combination of other drugs in the treatment of several neoplastic diseases.[5]Its immune modulatory effect it is use in the treatment of eczema. Methotrexate also used as a chemotherapy agent for decades, and selected for therapy of un-ruptured ectopic pregnancies.[6] And used in treatment of demyelinating polyneuropathy.[7] Methotrexate currently indicated in various pediatric cases like Hodgkin lymphoma, Osteosarcoma, brain tumors and meningeal leukemia.[8] Beta-carotene is a lipid soluble carotenoids and it has natural antioxidants pigment which is usually found in various vegetables and fruits. It is an originator of vitamin A with so much antioxidant and immune properties. It boost up the antioxidant defensive system by inhibiting the single oxygen and combating peroxide radicals, and reacting directly with Per oxy radical through stabilizing the lipids membrane from target of free radicals. Beta-Carotene (BC) obstructs and upgrades the effects of APAP on liver tissue. Many studies have shown that BC has anti carcinogenic properties. It inhibits the carcinogenic activity, genetic damage and caspase-3 activity. It reduces the risk of cancers.[9] The other antioxidant compound Resveratrol (3,4,5) Trihydroxistillbene (Rsv) (3,4,5 trihydrostillbene) is a bioactive compound found in a great variety of plants such as plums, blueberry, peanuts and grapes. Various studies were conducted on (Rsv) and suggests the benefits for healthy antioxidants, anti-Inflammatory and hepatoprotective effects. Regarding liver disease studies it is known that the proliferation of stellate cells which plays key role in liver injury aggravating via oxidative stress. For this effect the bioactive compound prevent the liver cell damage.[10]

MATERIALS AND METHODS

An experimental study was conducted on 48 healthy Wistar albino rats after attaining ethical approval from the institutional review board of the university. The study took place at Al-Tibri Medical College & Hospital from January 2021 for a period of October 2021. The subjects were acquired from the animal house of the institute weighting between 210-310 grams. All the animals were kept in clean cages in a well-ventilated room with adequate light and ambient temperature between 23-25◦C. food and water were available ad libitum. Methotrexate, B-carotene, and Resveratrol were purchased from a local pharmacy shop. The subjects were divided into 6 groups each consisting of 8 subjects. The following intervention was performed in each group:

- Group I (control) was administered similar amount of 0.9% Normal Saline Solution only. `
- Group II (□-carotene) was administered a solution of β-carotene (10mg/kg/ day intraperitoneally) for a period of 24 days.
- Group III (Resveratrol) rats received resveratrol (10mg/kg/ day intraperitoneal) for a period of 24 days.
- Group IV (MTX) rats were given MTX on the 21st day of the study as a one-time only injection intraperitoneally 20mg/kg.
- Group V (β-carotene + MTX) subjects were administered β-carotene by intraperitoneally (10 mg/kg/day) for a period of 24 days, and then on the 21st day of the experiment they were also given MTX at a dose level of 20 mg/kg.
- Group VI (Resveratrol + MTX) rats were given intraperitoneally (10mg/kg/day) for a period of 24 days, and then on the 21st day were given MTX at a dose level of 20 mg/kg on day.

On day 25th of the study, all the subjects were weighted and euthanized under anesthesia. A midline longitudinal incision was given to expose the organs... Liver tissues for Catalase were stored at -25◦C for quantitative analysis. Histological study was carried out at the institution. The liver extracted from the subjects was wash with normal saline and cut into small pieces, fixed into 10% formalin for PAS for 36 hours. The liver samples were then stained using H&E staining for histological study. The nuclear diameter was measured with ocular counting scale on a light microscope. The liver homogenate was used to analyze catalase enzyme content. The activity of catalase was measured at 37◦C by following the rate of disappearance of hydrogen peroxide at 240nm. SPSS was used to analyze the data. The results were compared with paired t-test with $p< 0.05$ considered as statically significant. All results were expressed as means ± standard error (SEM).

RESULTS

Table 1: Shows the Mean Nuclear Diameter (μm) of Each Group

Table 2: Shows the Statistical Comparison using Paired T-test of Nuclear Diameter among Various Groups I to VI

Table 3: Shows the Biochemical parameters Catalase (CAT) analyzed in liver homogenate of experimental animals (Albino rats = 250 Gm average), to assess the

hepatotoxicity of Methotrexate and its attenuation by -carotene and Resveratrol.

Table 4: Shows the Comparative statistical analysis of CAT of various Groups I to VI by paired T-test.

Table No.1: Nuclear Diameter of Hepatocytes (µm) in Group I – VI

Groups	Mean ± Standard Error Mean (SE)
Group I (Saline)	5.067 ± 0.1256
Group II (β-Carotene alone)	5.233 ± 0.0422
Group III (Resveratrol alone)	5.217 ± 0.0601
Group IV (Methotrexate alone)	5.733 ± 0.0803
Group V (β – Carotene + MTX)	5.483 ± 0.0307
Group VI (Resveratrol + MTX)	5.467 ± 0.0494

Results are expressed as Mean ± SE (Standard Error Mean = SE) MTX = Methotrexate

Table No.2: Statistical Comparison using Paired T-test of Nuclear Diameter among Various Groups I to VI

Paired Samples Statistics (95% Confidence Interval of the Difference)	
Paired Comparison Groups	Sig.(2-tailed) (P< 0.05)
Group I (Saline) vs. Group IV (Methotrexate)	.008**
Group II (-Carotene) vs Group IV (Methotrexate)	.002**
Group III (Resveratrol) vs Group IV (Methotrexate)	.006**
Group V (β – Carotene + MTX) vs Group IV (Methotrexate)	.048**
Group VI (Resveratrol + MTX) vs Group IV (Methotrexate)	.042**
Group V (β – Carotene + MTX) vs Group VI (Resveratrol + MTX)	.695*

Results were considered significant when P < 0.05
MTX = Methotrexate
KEY: ** = Significant * = non-Significant

Table No.3: Biochemical parameters Catalase (CAT) analyzed in liver homogenate of experimental animals (Albino rats = 250 Gm average), to assess the hepatotoxicity of Methotrexate and its attenuation by -carotene and Resveratrol

Biochemical parameters in liver homogenates (Mean ± SE)	
Groups	CAT (U/mg Protein)
Group I (Saline Control)	143.5 ± 3.21
Group II (-Carotene alone)	143.15 ± 2.87
Group III (Resveratrol alone)	149.17 ± 3.85
Group IV (Methotrexate alone)	92.71 ± 1.93
Group V (-carotene + MTX)	123.09 ± 3.19
Group VI (Resveratrol + MTX)	125.5 ± 1.95

Results are expressed as Mean ± Standard error of mean (SE).
MTX = Methotrexate, CAT = Catalase

Table No.4: Comparative statistical analysis of CAT of various Groups I to VI by paired T-test

Groups	Sigificance (2-tailed)
G-I vs G-II	0.11*
G-I vs G-III	0.09*
G-I vs G-IV	0.0001**
G-II vs G-V	0.03**
G-III vs G-VI	0.03**
G-IV vs G-II	0.001**
G-IV vs G-III	0.001**
G-IV vs G-V	0.01**
G-IV vs G-VI	0.01**
G-V vs G-VI	0.11*

G = group. Results were considered significant when P < 0.05
Statistical significance P < 0.0001, P < 0.001, P < 0.01, P < 0.03
KEY:** = Significant * = Non Significant

DISCUSSION

Methotrexate is a potent drug with multiple uses. Its association with the group of anti-folate and –anti-metabolite drugs allows it to be given in the treatment of leukemia, lymphoma, rheumatoid arthritis, and several other solid tumors. [11] Despite its many uses in medicine, side effects of high dose methotrexate can be life threatening. Side effects include renal toxicity, neurotoxicity, hematological toxicity, pulmonary toxicity, mucocutaneous toxicity, and gastrointestinal toxicity [12]. Drug interactions can also increase the risk of Methotrexate toxicity, as drugs such as sulfamethoxazole and Trimethoprim can all displace methotrexate from bound protein in the serum reducing methotrexate clearance. There for drugs which alter the rate of elimination of methotrexate must be recognized by the practitioner so that drug-drug interaction can be avoided. [13] The aim of our study was to see if β-carotene and Resveratrol can reduce the toxicity induced by Methotrexate. The study observed an increase in the nuclear diameter of hepatocytes in the methotrexate treated group. This was due to injury to the liver hepatocyte cells caused by methotrexate. [14] This can be explained on the basis of distortion and disintegration of hepatocytes architecture. Significant difference was observed in the mean nuclear diameter when all the groups were compared to the methotrexate only group. Our study showed that Resveratrol and β-carotene both prevented an increase in the mean nuclear diameter of hepatocytes thereby reducing the toxic potential of methotrexate. [15] This finding is similar to another study that showed that resveratrol protects against methotrexate induced hepatic injury.[16-17] Firdous et al (2011) also reported that β-carotene administration had a hepatoprotective effect against the generation of free radicals and reduced oxidative stress, thereby leading to a reported improvement in the hepatocyte architecture. A decrease in the amount of

catalase enzyme was seen in the methotrexate treated group with other groups showing a significant difference in the amount of catalase. Both the groups with Resveratrol and β-Carotene prevented a reduction in the amount of catalase enzyme when methotrexate was administered along with it. [18] This is similar to another study by Dalaklioglu (2013) which showed that resveratrol significantly improved the activity of catalase in liver when compared to methotrexate treated group only. [18] The increased level of catalase is also seen in another study by El-Demerdash et al (2004) by showing that β-carotene is a potent antioxidant that increases the level of Catalase. [19] Furthermore, it was reported by Cetin et al (2008) that Resveratrol reduces oxidative stress and increases the levels of Catalase. [21] Further studies can be done to study more histological and biochemical features that can be altered by the administration of Both Resveratrol and β-Carotene.[20]

CONCLUSION

According to the study results both therapeutic agents were significantly effective in prevention of the toxic effects of methotrexate on liver. There potent antioxidant effect was evidenced through morphological restoration of liver architecture and catalase activity.

Author's Contribution:

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Conflict of Interest: The study has no conflict of interest to declare by any author.

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