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ORIGINAL ARTICLE

Hepatotoxic Effect of Methotrexate on Serum Hepatic Enzymes with Amendment by Sulforaphane in Albino Rats

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ABSTRACT

Objective: To evaluate the variations in serum levels of hepatic enzymes in methotrexate damaged hepatic tissue with improvement by sulforaphane.

Design of research study: Experimental research study.

Duration of research study: this research study was piloted in BMSI, JPMC & total period was one week & three days.

Materials and Methods: For research work 40 adult albino rats of 0.2-0.3kg were selected. Time period for final study was 1 week and 3 days because animals started dying after 10 days due to hepatotoxic effect of methotrexate. Animals were separated into 4 groups, A1 was control, B1 animals were Injected Methotrexate intraperitoneally. C1 animals were Injected Methotrexate intraperitoneally along with sulforaphane by N/G. D1 animals was given sulforaphane by N/G. At the end of study, animals were sacrificed & blood was taken by direct cardiac puncture and send for lab investigation.

Results: B1 animals presented with significant increase in serum levels of hepatic enzymes while C1 animals had slightly raised serum levels of hepatic enzymes.

Practical Implication: These findings contribute further support to the hypothesis that variations in serum levels of hepatic enzymes in methotrexate damaged hepatic tissue are improved by sulforaphane administration.

Conclusion: Research accomplishes that sulforaphane altered the unfavorable effects of methotrexate. Group B1 had substantial raise in serum levels of hepatic enzymes whereas Group C1 serum had substantial decrease in serum levels of hepatic enzymes So our recommendation is that sulforaphane should be use with methotrexate to reduce its side effects.

Keywords: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), reactive oxygen species (ROS), methotrexate(MTX), glutathione (GSH), NADPH (nicotinamideadenine dinucleotide phosphate).

INTRODUCTION

Liver is the most important vital organ; detoxify chemicals, drugs and environmental pollutants injurious for hepatic tissue. A folic acid antagonist Methotrexate (MTX) is used as an anticancerous agent & for the management of rheumatoid arthritis, ectopic conception, psoriasis, autoimmune ailments and inflammatory conditions because it is a steroid-sparing immunosuppressant. [1, 2] Methotrexate causes oxidative stress-mediated injury by reducing folate species and alterations in various biochemical pathways by propagating reactive oxygen species (ROS) production and decrease in intracellular levels of NADPH, which causes reduction of glutathione (GSH) a significant cytosolic antioxidant agent.[3,4] Methotrexate exerts harmful effect on the mitochondria and endoplasmic reticulum thus causes raised reactive oxygen species levels (ROS) which damages cellular macromolecules, trigger lipid peroxidation and apoptosis.[5] It considerably raised the serum levels of AST, ALT, ALP and LDH, while reduces SOD activity.[6] It also causes liver steatosis, fibrosis and cirrhosis by producing superoxide anion, hydrogen peroxide and hydroxyl radicals.[7,8] It suppresses immunity thus causes low white blood cell count which leads to acute pneumonitis.[9,10] It also causes lung injury, renal injury, GIT disorders, alveolar fibrosis and mediator of autophagic cell death by activating Hematopoietic stem cells and pro-fibrogenic effects on liver tissue by converting Hematopoietic stem cells into myofibroblasts,[11] thus increasing inflammation and hepatic fibrosis and cirrhosis.[12,13] Due to alteration of antioxidant defenses lipid peroxidation initiated and inflammatory mediators like tumor necrosis factor-α (TNF-α) & nitric oxide synthase released which are key factors in hepatic tissue homeostasis.[14]

Sulforaphane is derivative of myrosinase by hydrolysis of glucoraphanin & commonly used as isothiocyanate organosulfur compound & phytochemical element of cruciferous vegetables, exhibiting multiple biological properties like it act as antipsoriasis, chemopreventive, antioxidant, antimicrobial, anticarcinogenic, antidiabetic agent.[15,16] It inhibits cell viability and induced DNA strand disruptions as well as activates nuclear factor & intracellular glutathione (GSH) levels thus reduces free radicals production & oxidative stress.[17,18] It is highly chemopreventive against colorectal carcinoma, urinary bladder carcinoma, prostate carcinoma, breast carcinoma, thyroid carcinoma.[19,20] Aging is a natural phenomenon occurs due to deterioration of cellular functions but Sulforaphane reduces lipotoxicity, glucotoxicity & hepatic fibrous through its antioxidant effects,[21] improves oxidative stress & helpful in attenuating metabolic disorders & shifts the balance of cancer-causing agent breakdown to deactivation. [22, 23] It is also beneficial for reducing obesity, diabetes, apoptosis, inflammation and reactive oxygen species production.[24,25,26]

Its hepatoprotective action has been well-documented, although the precise mechanism through which this occurs is still unclear. We are unaware of any studies evaluating sulforaphane's potential protective effects against MTX-induced hepatotoxicity. The current study aimed to better understand the effects of sulforaphane on the serum levels of hepatic enzymes and their variability in response to methotrexate-induced hepatotoxicity.

MATERIAL & METHOD

This research study was conducted in BMSI anatomy department, JPMC. 0.3-0.4 years old 40 adult rats of 0.2-0.3 kg were used in this research work, obtained from USA Lab and cross bred in animal house of JPMC. Initially the time period was 20 days but in pilot study animals expired after 1week & 3days, thus the time was reduced to 1week & 3days. Animals were retained in cages in an airy area. Animals were separated into 4 groups & observed for 7 days for any health issues. Afterwards animals were treated with inj.Methotrexate (injection unitrexate manufactured by Korea united pharm)[27]and cap. Sulforaphane (Green Food (Dietary supplement) capsule made by Swanson health product (USA).[28]
A1 was control, B1 animals were Injected Methotrexate intraperitoneally. C1 animals were Injected Methotrexate 5g/1000g intraperitoneally along with sulforaphane by N/G. D1 animals was given sulforaphane500µg/1000g by N/G.

A1: was control group on normal diet.
B1: Was Injected Methotrexate intraperitoneally.
C1: Was Injected Methotrexate intraperitoneally along with sulforaphane by N/G tube.
D1: were given only sulforaphane by N/G tube.
At the end of study, animals were sacrificed & 10ml blood was taken by direct cardiac puncture and send for lab investigation of enzymes (serum alanine amino transferase ALT and serum alkaline phosphatase ALP) to relate it with the cellular injury in hepatic tissue.

RESULTS

The serum analysis of liver enzymes, alanine aminotransferases (ALT) and alkaline phosphatase (ALP) was done for assessment of hepatic injury.

Observation on serum level of Alanine Aminotransfererase (ALT) (I.U/L)

Control Group-A1: The mean value of serum ALT level of control groups-A1 was 31.60±0.5 (Table-1).

Methotrexate treated Group-B1: The mean value of serum ALT level in group-B1 was 38.20±0.8. The data showed that there was highly significant increase (P<0.001) in serum ALT level of group-B1 in comparison to control group-A1 (Table-1).

Sulforaphane protected Group-C1: The mean value of serum ALT level of group-C1 was 34.00±0.70. A significant increase (P<0.05) in serum ALT level of group-C1 was observed in comparison to control group-A1. The data also showed moderately significant decrease (P<0.005) in serum ALT level of group C1 as compared with group-B1 animals (Table-1).

Observation on serum level of Alkaline Phosphatase (ALP) (I.U/L)

Control Group-A1: The mean value of serum ALP level in control groups-A1 was 144.58±0.65 (Table-2).

Methotrexate treated Group-B1: The mean value of serum ALP level in group-B1 was 300.40±0.54. The data showed highly significant increase (P<0.001) in serum ALP level of group-B1 in comparison to control group-A1 (Table-2).

Sulforaphane protected Group-C1: The mean value of serum ALP in groups-C1 was 147.00±0.70. A moderately significant increase (P<0.005) was observed in mean serum ALP level of group-C1 as compared to control group-A1 whereas a highly significant decrease (<0.001) was noticed in group C1 when compared group-B1 (Table-2).

Table-1 (a): Mean serum level of alanine aminotransferase (i.u/l) in different groups of albino rats

Groups	Treatment given	Serum level of ALT
A1 (n=10)	ND	31.60±0.5
B1 (n=10)	Inj. Methotrexate	38.20±0.8
C1 (n=10)	Inj. Methotrexate + Oral. Sulforaphane	34.00±0.7

Table -1 (b): Statistical analysis of the difference in mean serum level of Alanine Aminotransferase in different groups of albino rats

Statistical Comparison	P-value
B1 vs. A1	P<0.001****
C1 vs. A1	P<0.05**
C1 vs. B1	P<0.005***

Table-2 (a): mean serum level of alkaline phosphatase (i.u/l) in different groups of albino rats

Groups	Treatment given	Serum level of ALP
A1 (n=10)	ND	144.58±0.65
B1 (n=10)	Inj. Methotrexate	300.40±0.54
C1 (n=10)	Inj-Methotrexate + Oral.Sulforaphane	147.00±0.70

Key: Non-significant* significant**, Moderately-significant***, Highly-significant****, ND: Normal Diet

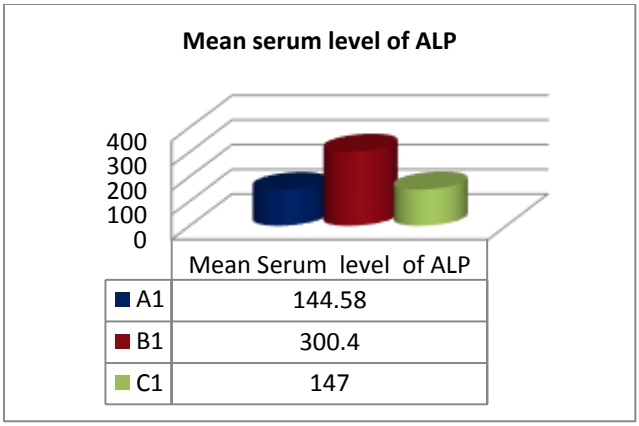

Figure: mean serum level of alkaline phosphatase (i.u/l) in different groups of albino rats

Table 2 (b): Statistical analysis of the difference in mean serum level of Alkaline Phosphates in different groups of albino rats

Statistical comparison	P-value
B1 vs. A1	P< 0.001****
C1 vs. A1	P < 0.005***
C1 vs. B1	P< 0.001****

Key: Non-Significant *, Significant**, Moderately-significant***, Highly-significant****, ND: Normal Diet

DISCUSSION

Methotrexate is a folic acid antagonist used as an anticancerous agent & for the management of rheumatoid arthritis, ectopic conception, psoriasis, autoimmune ailments and inflammatory conditions because it is a steroid-sparing immunosuppressant. [1,2] It exerts harmful effect on the mitochondria and endoplasmic reticulum thus causes raised reactive oxygen species levels (ROS) which damages cellular macromolecules, trigger lipid peroxidation and apoptosis.[5]It considerably raised the serum levels of AST, ALT, ALP and LDH, while reduces SOD activity.[6]

Sulforaphane is derivative of myrosinase by hydrolysis of glucoraphanin & commonly used as isothiocyanate organosulfur compound & phytochemical element of cruciferous vegetables, exhibiting multiple biological properties like it act as antipsoriasis, chemopreventive, antioxidant, antimicrobial, anticarcinogenic, antidiabetic agent.[15,16] It inhibits cell viability and induced DNA strand disruptions as well as activates nuclear factor & intracellular glutathione (GSH) levels thus reduces free radicals production & oxidative stress.[17,18]

B1 rats had elevated serum levels of hepatic enzymes which showed hepatotoxicity because of oxidative stress-mediated cellular injury by depleting folate species thus enhances purine breakdown as explain by.[1,2,3]

While group-C1 animals showed reduced serum levels of hepatic enzymes as compare to animals of group-B1. Because Sulforaphane act as chemopreventive, antioxidant, antimicrobial, anticarcinogenic, antidiabetic agent as explain by.[15,16] It inhibits cell viability and induced DNA strand disruptions as well as activates nuclear factor & intracellular glutathione (GSH) levels thus reduces free radicals production & oxidative stress as explain by.[17,18]

According to the present investigation, MTX triggered elevated levels of ALT & ALP activities. These findings corroborate the findings of Tunali-Akbay et al., who showed that MTX treatment in rats caused a toxic impact on liver function, as evidenced by a significant increase in serum activity of ALT and ALP. 29 Hepatocyte cytosolic enzyme ALT has been linked to cell death due to its association with an increase in serum activity. The alanine aminotransferase (ALT) is one of the most reliable markers of liver necrosis. 29 There are numerous accounts of how MTX caused hepatotoxicity. Certain amino and nucleic acid synthesis is inhibited because MTX may attach to the enzymes hydrofolic

reductase, as shown by Jahovic et al.30 and Al-motabagani et al.31. This might lead to destruction of mitochondria and cell membrane of hepatic endothelium disrupting with their activity and permitting leakage of enzymes. Methotrexate has been linked to tissue damage, and it has been hypothesized that this is because of the increase in MDA content and myeloperoxidase (MPO) levels that MTX causes. MDA and MPO are known to be key causes of degradation and damage to cell membranes.

The current study has notable limitations, including the relatively short length of Sulforaphane administration, which may have resulted in a more robust protective benefit with longer-term use of Sulforaphane therapy. Moreover, a small dose of MTX has been evaluated, despite the fact that the drug is often used as part of treatment regimens for long-term conditions. However, experimenting with MTX at varying doses over longer time periods is an area that needs more study. Furthermore, we were unable to further explore the impact of MTX or Sulforaphane upon that inflammatory process due to a lack of resources. To confirm our findings, additional research into the effects of Sulforaphane on the other molecular processes suggested by the injection of MTX in albino rats is required.

CONCLUSION

Research accomplishes that sulforaphane altered the unfavorable effects of methotrexate. Group B1 had substantial raise in serum levels of hepatic enzymes whereas Group C1 serum had substantial decrease in serum levels of hepatic enzymes So our recommendation is that sulforaphane should be use with methotrexate to reduce its side effects.

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