

# Pathological Impact of Methotrexate on Body Weight, Absolute and Relative Weight of Liver with Amelioration by Sulforaphane in Albino Rats

SADIA SUNDUS<sup>1</sup>, KANEEZ FATIMA<sup>2</sup>, TALAT SAMREEN<sup>3</sup>, ASMA AIJAZ<sup>4</sup>, ERUM KHAN<sup>5</sup>, KHALIDA ANWAR<sup>6</sup>

<sup>1</sup>Associate Professor, Department of Anatomy Bhawalpur Medical College, Bhawalpur

<sup>2</sup>Assistant Professor, Department of Anatomy Bolan Medical College, Quetta.

<sup>3,4</sup>Assistant Professor, Department of Anatomy Karachi Medical & dental College, Karachi.

<sup>5</sup>Assistant Professor, Department of Community Medicine Karachi Medical & dental College, Karachi

<sup>6</sup>Assistant Professor, Department of Biochemistry RYK Medical College & Allied Teaching Hospital

Correspondence author: Sadia Sundus, Email: [usadsun\\_dr@yahoo.com](mailto:usadsun_dr@yahoo.com), Cell: 0092-300-2850489

## ABSTRACT

**Objective:** To assess the changes in Body Weight, Absolute and Relative weight of Liver in methotrexate impaired liver with amendment by sulforaphane.

**Design of research:** Experimental.

**Abode and Period of study:** Research was conducted in BMSI, Karachi & duration was ten days.

**Materials and Methods:** For experiment Forty young albino rats of 3-4 months old of 200-300gm were taken, Primarily period of study was 20 days however during the pilot study, rats started dying after 10 days, so the duration was reduced to 10 days for final study. Rats were alienated into 4 sets, A was control group, B set was given Inj Methotrexate intraperitoneally. C set was given Inj Methotrexate intraperitoneally along with sulforaphane by N/G tube. D set was given only sulforaphane by N/G tube. After the completion of study, rats were dissected and liver was removed from abdominal cavity of rats and absolute weight of liver was weighed on Sartorius balance. The relative weight of the liver was calculated with the help of formula.

Mean weight of liver (G)

The relative weight of liver =  $\frac{\text{Mean weight of liver (G)}}{\text{Final weight of the animal}} \times 100$

Final weight of the animal

**Results:** B group showed remarkable decrease in the body weight while absolute liver weight & relative liver weight is increased however group C had slight reduction in the body weight while absolute liver weight & relative liver weight is slightly increased.

**Conclusion:** This study accomplishes that sulforaphane amended the detrimental effects of methotrexate.

**Keywords:** sulforaphane, methotrexate, superoxide dismutase (SOD), dihydrofolate reductase enzyme (DHFR), nuclear factor erythroid-derived 2(Nrf2), Tetrahydrofolate (THF)

## INTRODUCTION

Liver is a vital organ and detoxifying agent of body. Methotrexate is a preventer of dihydrofolate reductase, an antimetabolite and folic acid antagonist. It prevents the production of DNA, RNA, thymidylate and amino acids by increasing reactive oxygen species synthesis which causes mitochondrial dysfunction and endoplasmic reticulum hassle thus causing hepatic injury by inhibiting proliferation, differentiation, initiates inflammatory reactions & arbitrates autophagic apoptosis.<sup>1,2</sup> Likewise, a decrease in intracellular levels of NADPH diminishes cytosolic antioxidant agent glutathione. It is commonly used for management of autoimmune ailments, malignant lumps, SLE, rheumatoid arthritis, psoriasis and inflammatory conditions.<sup>3,4</sup> It reduces the replication of bone marrow and gastrointestinal epithelial cells thus causing leucopenia and thrombocytopenia, anemia and weight loss.<sup>5,6</sup> It causes hepatic injury due to apoptosis of hepatocytes, therefore the serum level of liver enzymes raised & reduces superoxide dismutase activity (SOD).<sup>7,8</sup> Reactive oxygen species produced which causes peroxidative damage to vital organs.<sup>9,10</sup> Methotrexate is also used for the treatment of malignancy by chemotherapy & act as cytotoxic anti-neoplastic and immunosuppressive agent.<sup>11</sup> It inhibits conversion of dihydrofolate (DHF) to tetrahydrofolate (THF) by preventing dihydrofolate reductase enzyme activity (DHFR) thus causing folic acid inhibition. Tetrahydrofolate (THF) is essential for the production of both purines and pyrimidines production.<sup>12,13</sup> Methotrexate also inhibits binding of interleukin-1 beta to cell surface receptors and deactivate B-cells and T-cell, so it is used in rheumatoid arthritis therapy.<sup>14,15,16</sup> It causes hepatic injury by production of free radicals & proinflammatory cytokines and damage normal body cells along with malignant cells.<sup>17,18,19</sup>

Sulforaphane (1-isothiocyanato-4-(methylsulfanyl)butane) is a phytochemical component of cruciferous vegetables like broccoli, cabbage etc and has antidiabetic, antimicrobial, antioxidant, neuroprotective, anticarcinogenic properties because it stimulates NADPH Quinone reductase, glutathione reductase & cytoprotective genes production and decreases inflammatory cytokines secretion.<sup>20,21</sup> It protect liver cells by stimulation of Nrf2 signaling & annulment of free radicals

and oxidative stress, reciprocally in vivo and in vitro.<sup>22</sup> Sulforaphane improves chronic inflammation by targeting macrophages and raises hepatic enzyme 3 $\alpha$ -hydroxysteroid dehydrogenases. It restores cognitive function in cirrhotic hepatic encephalopathy patients.<sup>23,24</sup> It detoxify reactive oxygen species through conjugation reactions thus reduces hepatocellular injury.<sup>25,26</sup> It promotes antioxidants enzyme activities & ameliorates oxidative stress as well as reduces hepatic fibrosis.<sup>27,28</sup> It also protects hepatic cells against lipopolysaccharide induced hepatic injury & prevents obesity.<sup>29,30</sup> In our knowledge, no research was designed to accomplish therapeutic effect of sulforaphane on body weight, absolute and relative weight of liver in methotrexate impaired liver.

## MATERIAL & METHOD

Research was conducted in anatomy department of Basic medical sciences institute, (JPMC), Karachi. 3-4 months old forty albino rats of 200-300gm were included in this study, which were obtained from USA Charles River Breeding Laboratories and were cross bred in animal house of BMSI. Primarily the period of study was 20 days but during the pilot study rats started dying after 10 days, so the duration was reduced to 10 days. Rats were weighed numbered and kept in cages in a well-ventilated room with 12 hours dark and light cycle. They were divided into four groups A, B, C, and D & each group comprised of ten animals. The animals were housed for one week before the start of study, for adaptation to the environment, assessment of their intake of normal diet, general health and behavior status.

After one week they were treated with calculated amount of injection Methotrexate and capsule sulforaphane. Each animal was weighed by using a triple beam balance before the commencement of study and at the time of sacrifices and tabulated.

In this study Methotrexate was used in the form of injection unitrexate made by Korea united pharm, Inc and Sulforaphane was used in the form of Green Food (Dietary supplement) capsule manufactured by Swanson health product (USA). Calculated amount of Inj Methotrexate, 5mg/kg body weight was given

intraperitoneal<sup>31</sup> and sulforaphane 500µg/kg body weight<sup>32</sup> was given orally through N/G tube to the experimental animals.

A served as control.

B were given Inj Methotrexate intraperitoneal.

C were given Inj Methotrexate intraperitoneal along with sulforaphane orally by N/G tube.

D were given only sulforaphane orally by N/G tube

The animals were sacrificed at the end of experimental period. Ether anesthesia was given in a glass container and then animals were fixed to the dissecting board. A midline longitudinal incision from the manubrium sterni to lower abdomen was given. Thoracic and abdominal viscera were exposed by careful removal of skin, fascia and muscles.

The liver was exposed by incising the diaphragm, gross morphological features of liver were observed for any change in color, size, contour, hemorrhage, pathology and any adhesion to the surrounding tissue, then the liver was removed and absolute weight of liver were weighed on Sartorius balance. The relative weight of the liver was calculated with the help of formula:

Mean weight of liver (G)

The relative weight of liver = X100

Final weight of the animal

**RESULTS**

This experimental study was designed to observe the effects of Methotrexate induced hepatic damage and the protective role of sulforaphane on Methotrexate induced-hepatotoxicity.

**Observations of Body Weight (gm)**

**A** The mean initial body weight in group-A was 209.40±2.61gm and mean final body weight in the same group was 210.40 ±2.0gm. The data showed no significant increase (P>0.05) in final body weight of group-A animals when compared with the initial body weight of same group (Table-1).

**B** The mean value of initial body weight of group-B was 226.60±3.1 gm and mean value of final body weight in group-B was 171.80±1.78 gm. There was highly significant decrease (P<0.001) in the final body weight of group-B when compared with initial body weight of same group. The data also showed highly significant decrease (P<0.001) in the final body weight of group-B, when compared with control group-A (Table-1).

**C** The mean value of initial body weight of sulforaphane protected group-C was 208.0±3.72 gm and the mean value final body weight of group-C was 207.0±2.1 gm. The data showed significant decrease (P<0.05) between initial and final body weight in group-C animals. There was also a moderately significant decrease (P<0.005) in final body weight of group-C observed in comparison with control group. A highly significant increase (P<0.001) in final weight of group-C was noticed when compared to final body weight of group-B animals (Table-1).

**Observations of Absolute Liver Weight (gm)**

**A** The mean value of absolute liver weight of groups-A was 5.76±0.37gm (Table-2).

**B** The mean value of absolute liver weight in group-B was 9.40±0.14 gm. The data showed, a highly significant increase (P<0.001) in absolute liver weight of group-B animals as compared to control group-A animals (Table-2).

**C** The mean value of absolute liver weight in groups-C was 7.94±0.5gm. A significant increase (P<0.05) in absolute liver weight of animals in group-C was observed as compared to control group-A. The data also showed moderately significant decrease (P<0.005) in absolute liver weight of group-C as compare to group-B (Table-2).

**Observation of Relative Liver Weight (gm)**

**A** The mean value of relative liver weight of control groups-A was 2.39±0.12gm (Table-3).

**B** The mean value of relative liver weight in group-B was 5.30±0.48gm. The data showed a highly significant increase (P<0.001) in relative liver weight of group-B as compared to control group-A (Table-3).

**C** The mean value of relative liver weight in group-C was 3.67±0.22 gm. There was a significant increase (P<0.05) in relative liver weight in group-C when compared to control group-A animals. The data also showed moderately significant decrease (<0.005) in relative weight in group-C as compared with group-B (Table-3).

Table-1: Mean Body Weight (G) in Different Groups of Albino Rats

Groups	Treatment given	Initial body weight	Final body weight
A (n=10)	ND	209.40± 2.61	210.40± 2.0
B (n=10)	Inj. Methotrexate	226.60± 3.1	171.80± 1.78
C (n=10)	Inj. Methotrexate + Oral. Sulforaphane	208.0± 3.72	207.0 ± 2.1

\*Mean±SEM

Statistical Analysis of the difference in mean body weight within the same group and between different experimental groups of Albino Rats

Statistical comparison	P-value	Statistical Comparison	P-value
Initial & final weight of A	P>0.05*	B vs. A	P<0.001****
Initial & final weight of B	P<0.001****	C vs. A	P<0.005***
Initial & final weight of C	P<0.05**	C vs. B	P<0.001****

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

Table 2: Mean Absolute Weight of Liver (G) in Different Groups of Albino Rats

Groups	Treatment given	Mean Absolute liver weight at the end of experiment
A (n=10)	ND	5.76 ± 0.37
B (n=10)	Inj. Methotrexate	9.40 ± 0.14
C (n=10)	Inj. Methotrexate + Oral. Sulforaphane	7.94 ± 0.5

\*Mean±SEM

Statistical Analysis of the difference in mean absolute weight of liver within the same group and between different experimental groups of Albino Rats

Statistical comparison	P-value
B vs. A	P<0.001****
C vs. A	P<0.05**
C vs. B	P<0.005***

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

Table-3: \*Mean Relative Weight of Liver (G/100g) in Different Groups of Albino Rats

Groups	Treatment given	Mean relative weight of liver at the end of experiment
A (n=10)	ND	2.39 ± 0.12
B (n=10)	Inj. Methotrexate	5.30 ± 0.48
C (n=10)	Inj. Methotrexate + Oral. Sulforaphane	3.67 ± 0.22

\*Mean±SEM

Statistical analysis of the difference in mean relative weight of liver within the same group and between different experimental groups of Albino Rats

Statistical comparison	P-value
B vs. A	P<0.001****
C vs. A	P<0.05**
C vs. B	P<0.005***

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



Figure 1: Gross Appearance of Control Rat Liver Showing Normal Color Size, and Smooth Surface of it.

**DISCUSSION**

Methotrexate is a preventer of dihydrofolate reductase, an antimetabolite and folic acid antagonist. It prevents the production of DNA, RNA, thymidylate and amino acids by increasing reactive oxygen species synthesis which causes mitochondrial dysfunction and endoplasmic

reticulum hassle thus causing hepatic injury by inhibiting proliferation, differentiation, initiates inflammatory reactions & arbitrates autophagic apoptosis.<sup>1,2</sup>

Sulforaphane (1-isothiocyanato-4-(methylsulfinyl) butane) is a phytochemical component of cruciferous vegetables like broccoli, cabbage etc and has antidiabetic, antimicrobial, antioxidant, neuroprotective, anticarcinogenic properties because it stimulates NADPH Quinone reductase, glutathione reductase & cytoprotective genes production and decreases inflammatory cytokines secretion.<sup>20,21</sup>

All the group B animals became lethargic and lost their body weight. This loss of body weight could be due to the intestinal mucositis and anti-mitotic or apoptotic activity of the methotrexate, because chemotherapeutic cytotoxic agents are not specific to tumor cells but they also induce deleterious effect on normal cells of the body as explain by.<sup>2,5,6</sup>

Whereas the Sulforaphane protected group-C animals were active and lost less body weight in comparison of group-B animals. This may be due to the fact that Sulforaphane prevents oxidative damage to the tissue and reduces apoptosis of cells as suggested by<sup>28,30</sup>

## CONCLUSION

This study accomplishes that sulforaphane amended the detrimental effects of methotrexate. Group B had significant decrease in body weight while Group C exhibited increase in body weight. So this is our suggestion use sulforaphane along with methotrexate to decrease hepatic injury.

**Conflict of Interest:** None

**Financial Disclosure:** None

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