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EFFECT OF METHOTREXATE INDUCED OXIDATIVE STRESS IN THE LIVER OF WISTAR ALBINO RATS

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Abstract: The present study was conducted to assess methotrexate (MTX) induced toxicity in Wistar male albino rats. The study had two groups with 12 rats in each. MTX was used at the dose of 5mg/kg b.w. to induce toxicity. In MTX control group the levels of endogenous antioxidant enzymes such as SOD, CAT and GPx were significantly reduced compared to those of normal control. The MDA levels caused by lipid peroxidation as a result of oxidant injury to liver was significantly higher in MTX positive controlthan compared to that of normal control. In conclusion MTX induces hepatotoxicity in rats through oxidative stress evidenced by decreased levels of endogenous antioxidant enzymes such as SOD, CAT and GPx and increased levels of MDA indicating increased extent of oxidative injury caused by MTX on hepatocytes.

Keywords: Methotrexate, oxidative stress, oxidative stress, endogenous anti oxidant enzymes.

1. INTRODUCTION

Methotrexate (MTX), a folic acid antagonist, currently used as a disease modifying anti-rheumatic drug (DMARD) along with non-steroidal anti-inflammatory drugs (NSAIDs) to provide the best possible relief to rheumatoid arthritis patients in low doses (2.5 mg–15mg/week) (Kremer *et al*.,1986). Rheumatoid arthritis is a chronic, systemic, autoimmune, inflammatory disorder of humans and animals like dog and horse which primarily affects joints resulting in non-suppurative proliferative synovitis. Methotrexate is also used alone or in combination with other anticancer agents in the treatment of breast cancer, epidermoid cancers of the head and neck and lung cancer.

Both low and high-dose therapy can cause hepatotoxicity (Saka *et al.,* 2017). Methotrexate at very low dosage affects the liver and causes changes in histology of liver. Other frequently reported adverse toxic effects are malaise, nausea, vomiting, diarrhoea, headache, mild alopecia and fever. Since MTX induces toxicity through systemic oxidative stress, a search

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for drugs or agents with antioxidant property has been extensively carried out (Ozogul *et al*., 2013), most of which proved partially helpful in preventing MTX toxicity.

2 MATERIALS AND METHODS

2.1 Experimental animals

Twenty four male Wistar Albino rats were procured from Biogen laboratory animal facility, Attibele, Bangalore, of the age group 8 to 10 weeks, with an average live weight of 180- 200 g. They were housed in cages and allowed to acclimatize under ambient temperature and standard daily light. They were given a nutritionally adequate specified rat's diet and water ad libitum throughout the experimental period. The experimental procedures were carried out according to the CPCSEA and approved by the Institutional Animal Ethics Committee.

Methotrexate, as Folitrax-15 injection was procured from IPCA laboratries, Mumbai, India. Other chemicals used are of commonly used laboratory grade.

2.2 Experimental design

After procurement, the rats were maintained under standard laboratory conditions for a period of 15 days for acclimatization in the experimental animal house. The rats were divided, based on the body weight, into two groups with twelve rats in each group. Group I was normal control, administered with PBS intraperitonealy for three consecutive and observed till 45 days. Group II were administered methotrexate at the dose rate of 5 mg/kg b.w. intraperitoneally with over night fasting for three consecutive days and observed for 45 days.

2.3 Collection of serum samples

Animals were sacrificed at 7^{th}, 21^{st} and 45^{th} day post induction of MTX toxicity, using over dose of Ketamine hydrochloride intraperitoneally. Liver of both group animals were collected in chilled normal saline and then transferred to -80^{o}C for further analysis.

2.4 Antioxidant parameters analysed

Endogenous antioxidant parameters such as catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) were assessed from the liver samples. To assess the oxidative injury to hepatocytes levels of malondialdehyde (MDA) was estimated.

3 RESULTS AND DISCUSSION

In the present study the effect of methotrexate induced hepatotoxicity on endogenous antioxidant enzymes in the liver of rats and the extent of oxidative injury to hepatocytes was analysed. The results showed a significant($P<0.05$) lower levels of CAT, GPx and SOD (Table 1, 2 and 3) and there was a significant ($P<0.05$) increase in the MDA levels in all the intervals of observation in comparison with normal control (Group 1) whose values

remained in the normal range throughout the experiment.

Table 1: Mean ± SE values of Catalase (CAT) levels (µmol/min/mgprotein) in rat liver of different groups at different time intervals

Groups	7th day	21st day	45th day
Group I (Control)	47.18 ± 0.418^{ax}	51.588 ± 0.902^{ay}	51.612 ± 0.448^{ay}
Group II (MTX)	18.845 ± 0.180^{bx}	21.883 ± 0.399^{by}	27.677 ± 0.522^{bz}

Values with different superscripts in a rows and columns vary significantly at P<0.05

Table 2: Mean ± SE values of glutathione peroxidase (GPx) levels (U/mg protein) in rat liver of different groups at different time intervals

Groups	7th day	21st day	45th day
Group I (Control)	35.361 ± 1.918^{ax}	36.410 ± 1.957^{ax}	35.6880 ± 1.672^{ax}
Group II (MTX)	9.443 ± 0.239bx	13.340 ± 18.768by	13.340 ± 18.768^{bz}

Values with different superscripts in a rows and columns vary significantly at P<0.05

Table 3: Mean ± SE values of superoxide dismutase (SOD) levels (U/min/mg protein) in rat liver of different groups at different time intervals

Groups	7th day	21st day	45th day
Group I (Control)	31.872 ± 1.625^{ax}	30.630 ± 1.248^{ax}	32.317 ± 0.989^{ax}
Group II (MTX)	8.845 ± 0.614^{bx}	11.730 ± 0.637^{bx}	17.112 ± 1.136^{by}

Values with different superscripts in a rows and columns vary significantly at P<0.05

Table 4: Mean ± SE values of malondialdehyde (MDA) levels (mmoles/mg of tissue) in rat liver of different groups at different time intervals

Groups	7th day	21st day	45th day
Group I (Control)	0.923 ± 0.012^{ax}	0.980 ± 0.018^{ax}	1.00 ± 0.024^{ax}
Group II (MTX)	5.708 ± 0.344^{bx}	7.015 ± 0.320^{by}	4.644 ± 0.234^{bz}

Values with different superscripts in a rows and columns vary significantly at P<0.05

The decrease in antioxidant enzyme levels such as CAT, GPx and SOD and increase in MDA levels in methotrexate administered animals was also observed by earlier workers (Jahovic *et al*., 2003, Deepak *et al*., 2015 and Vijaykumaar, 2016).

The free radicals and reactive oxygen species are formed physiologically by the oxidation and reduction reactions of various biomolecules involved in various cellular processes. The sources of physiological free radicals (FR) and reactive oxygen species (ROS) include

mitochondria, cyt-p450, NADPH oxidase and xanthane dehydrogenase/oxidase. Cellular reduced glutathione is the most important and efficient antioxidant present in the cells to combact physiological oxidative stress. (Saka *et al*., 2017) by harvesting the free radicals and ROS. When the levels of ROS or FRs production is more than the capacity of reduced glutathione to harvest them it leads to oxidative stress. Oxidative stress is produced exogenously by many chemotherapeutic drugs also and MTX is also one of them (Mianzono *et al*., 2013; Nema *et al*., 2019).

Oxidative cellular damage with profound lipid peroxidation are hallmarksof MTX toxicity (Cetin *et al*., 2008, Hany *et al.,* 2017). Methotrexate inhibits cytosolic NADP dependent dehydrogenases and NADP malic enzymes which reduces availability NADPH to maintain the reduced state of glutathione in the cell, which can act as scavenger of ROS and FR and there by reducing the lipid peroxidation caused by the ROS. Hence cell antioxidant defense system fails to handle the oxidative load leading to decreased anti oxidant enzyme levels such as catalase (CAT), glutathione peroxidase (GPx), which converts unstable hydrogen peroxide molecule into water and oxygen; and super oxide dismutase (SOD) which scavenges hydroxyl radicals. MDA levels, which is a end product of lipid peroxidation of cell and cellular membranes stimulated by the ROS overload caused by MTX leading to cell injury (Kumari, 2016, Tian *et al*., 2007).

Conclusion

Methotrexate at 5mg / kg b.w. intraperinonealy for three consecutive days induces oxidative stress by decreasing the cellular endogenous antioxidants enzymes such as CAT, GPx and SOD and thereby increases the MDA levels by causing oxidative injury to cells.

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