

Original Research Article

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Effect of Zinc Oxide Nanoparticles (ZnO NP) on Antioxidant Status of Methotrexate (MTX) Induced Toxicity in Wistar Albino Rats

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ABSTRACT

The experiment was undertaken to evaluate the effect of zinc oxide nanoparticles (ZnO NPs) on antioxidant status of methotrexate intoxicated Wistar albino rats. Seventy-two rats of 180 to 200g weight were equally divided into six groups. MTX was used at the rate of 5mg/kg b.w intraperitoneally for three consecutive days to induce toxicity and ZnO NPs was used @ of 50mg/kg b.w through oral gavage for 45 days. Antioxidant status of rats was evaluated by estimating products of oxidative injury (MDA) and endogenous antioxidant enzymes (CAT, SOD and GPx) in the liver on 7th, 21st and 45th days of experiment. All the antioxidant enzymes markedly reduced in MTX positive control with increased levels of MDA. In ZnO NPs positive control, the enzymes were numerically higher than normal control with mild increase in MDA levels at later stages of experiment. The MTX and ZnO NPs treated rats showed improved antioxidant status and decreased MDA levels than MTX alone intoxicated rats. ZnONPs pre-treated rats showed improved antioxidant profile than ZnO NPs concurrent treatment and were comparable with that of a proven herbal hepato-protectant 'Silymarin'. In conclusion, the experiment suggested that implementation of ZnO NP can combat MTX toxicity.

Keywords

Methotrexate, Zinc oxide nanoparticles, Endogenous antioxidant enzymes, Malondialdehyde, Oxidative injury

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Introduction

For the desirable health improvement and extended life span in certain disease conditions there is an inevitable need for chronic use of drugs. Autoimmunity and neoplasia are among the disease conditions

that require chronic use of chemotherapeutic drugs. Folate antagonists were among the first antineoplastic agents to be developed and aminopterin is one among them.

In 1948, aminopterin was used to induce remission in childhood acute lymphoblastic

leukemia (ALL), and the related agent methotrexate (MTX, 4-amino-N10-methyl folic acid) is still an important component of modern treatment for acute lymphoblastic leukaemia as well as a number of other hematologic malignancies (Nencini *et al.*, 2007). MTX was the first drug shown to cure cancer when given as monotherapy and still remains as a cornerstone of treatment for malignant gestational trophoblastic disease (Moshtaghion *et al.*, 2013). MTX is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines, needed in a health system (WHO, April 2015). The undesirable effects of this drug are mainly attributed to its ability to induce marked oxidative stress. Hence there is urge to discover suitable antioxidants that can be implemented in combating such drug induced toxicities.

In the recent past, the rapid development of nanotechnology has contributed to the production and control of engineered nanoparticles, which are generally defined as particles in the size range of 1-100 nm (Baek *et al.*, 2012). The small particle size of NPs creates a large surface area per unit mass and makes them more reactive in a cell (Donaldson *et al.*, 2005).

Zinc is a biodegradable, biocompatible metal and is an indispensable trace element. For adults 8–15 mg of zinc per day is recommended, 11mg for male and 9 mg for female of which, approximately 5–6 mg/day is lost through urine and sweat (Khwaja *et al.*, 2018). ZnO NPs are extensively used in cosmetics and sunscreens because of their efficient UV absorption properties, in the food industry as additives and in packaging due to their antimicrobial properties. They are also being explored for their potential use as fungicides in agriculture and imaging in biomedical applications (Rasmussen *et al.*, 2010). Zinc oxide nanoparticles are used as an

adjuvant treatment to alleviate the toxic effects of chemotherapeutic drugs (Elshama *et al.*, 2018). In the present study the ZnONP are evaluated for their ameliorative effect on MTX induced oxidative stress, in comparison with a proven herbal hepato-protectant 'Silymarin' in Wistar albino male rats.

Materials and Methods

Wistar albino male adult rats of 6-8 weeks weighing approximately 180 to 200g were procured from Committee for the purpose of control and supervision of experiments on animals (CPCSEA) approved laboratory animal vendor and housed in the laboratory animal facility of the institution with standard day light and dark cycle. The animals were provided with *ad libitum* feed and water. Standard rodent feed was procured from Indian Immunologicals, Hyderabad.

Following the acclimatization period of 10 days, the laboratory animals were divided into six groups with 12 rats in each. Group I was kept as normal control, Group II was MTX positive control, Group III was ZnO NP control, Group IV was ZnO NP pre-treatment where rats were treated with ZnO NP 14 days prior to induction of MTX toxicity, Group V was rats containing MTX induced toxicity and concurrently treated with ZnO NP for 45 days. Group VI contained rats with induced MTX toxicity and concurrently treated with proven herbal hepato-protectant 'silymarin' at the dose rate of 200mg/kg b.w. orally. MTX toxicity was induced at the rate of 5 mg/kg b.w. intraperitoneally after overnight fasting for three consecutive days. ZnONP was administered at the rate of 50 mg/kg b.w. for 45 days.

Three animals were sacrificed from all the groups on 7th and 21st day, and remaining six animals from each group on 45th day post induction of MTX toxicity. The liver samples

were collected in chilled normal saline and stored in -80°C.

Malondialdehyde in the liver samples collected from rats of different groups were estimated by the method as described by Yagi (1976). The endogenous antioxidant enzymes CAT, SOD and GPx were estimated by the methods as described by Claiborne (1985), Marklund and Marklund (1974) and Rotruck *et al.*, (1973) respectively.

Results and Discussion

In the present study the MDA levels of methotrexate control (Group II) in liver tissue was significantly ($P < 0.05$) higher than normal control (Group I) and also all other treatment groups throughout the experimental period. The level of MDA in ZnO NPs pre-treatment group was significantly ($P < 0.05$) lowered which was comparable with that of silymarin treatment group and lesser than that of ZnO NPs concurrent treatment group throughout the experimental period.

The levels of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were significantly ($P < 0.05$) reduced in the MTX control when compared to control and all other treatment groups. The enzymes levels in the ZnO NPs control were marginally higher than those of normal control throughout the experiment. The antioxidant enzymes levels in the ZnO NPs pre-treatment group were comparable with silymarin co-treatment group and significantly ($P < 0.005$) higher than ZnO NPs concurrent treatment group on all the intervals of examination.

Oxidative stress is a metabolic dysfunction that favours the oxidation of biomolecules, contributing to the oxidative damage of cells and tissues. MDA is the final product of lipid peroxidation and its level indicates level of oxidative damage to the organ.

Glutathione is present in the cytoplasm of cells and in its reduced state it is an antioxidant required to provide protection against ROS by utilization of NADPH by glutathione reductase (Babaik *et al.*, 1998). MTX inhibits cytosolic NADPH (reduced nicotinamide adenine dinucleotide phosphate) and NADP (Nicotinamide adenine dinucleotide phosphatase) enzymes and decreases the availability of NADPH in cells, thus subjecting the cells for oxidative stress/ROS injury.

In the present study following treatment with ZnO NPs a significant decrease in the level of MDA was observed in both the groups (IV and V) indicating the protective effect of ZnO NPs against MTX toxicity which could be attributed to the antioxidant property of Zinc. It protects cells against oxidative damage by stabilization of membranes. Zinc competes with iron and copper in the cell membrane and inhibits the enzyme nicotinamide adenine dinucleotide phosphate oxidase (NADPH-Oxidase), a pro-oxidant enzyme, and also induces metallothionein synthesis.

Zinc is bound to metallothionein under normal physiological conditions. In oxidative stress conditions, the micronutrient is released from its complex with metallothionein and is redistributed in the cells to exert antioxidant actions (Maret and Krezel, 2007 and Pzcelik and Naziroglu, 2012). Metallothionein is an oxidative stress-buster involved in the reduction of hydroxyl radicals (OH) and in the sequestration of the reactive oxygen species produced under stress conditions (Chasapis and Loutsidou, 2012 and Ruz and Carrasco, 2013).

Zinc also influences important enzymes that contribute to the proper functioning of the antioxidant defense system. The present study reported a significant ($P < 0.05$) increase in the levels of SOD, catalase and GPx in ZnO NPs

treated groups. Zinc is a structural component of the enzyme superoxide dismutase (SOD) present in the cytoplasm of cells.

Superoxide dismutase has an active centre with a copper ion and a zinc ion. This enzyme promotes the conversion of two superoxide radicals to hydrogen peroxide and molecular oxygen, reducing the toxicity of ROS as it converts a highly reactive species to a less harmful one (Cruz and Soares, 2011).

SODs and catalase are metalloproteins which catalyze “dismutation” reactions and detoxify O_2^- and H_2O_2 , respectively. SODs catalyze the formation of oxygen and H_2O_2 from two O_2^- , whereas catalase catalyzes the formation of oxygen and water from two H_2O_2 molecules (Biran, 2009).

Another mechanism by which zinc acts as an antioxidant is by affecting the expression of glutamate-cysteine ligase, which is a rate-limiting enzyme of glutathione de novo synthesis. This has a two-fold effect of zinc to neutralize free radicals directly by glutathione or indirectly as a glutathione peroxidase cofactor (Eide, 2011).

Inhibition of lipid peroxidation and improvement in antioxidant levels by ZnO NPs was also observed earlier by Alkaladi *et al.*, (2014) in diabetic rats and Grungriff, (2002) reported that ZnO NP treatment elevate antioxidant levels and thus maintains the cell membrane integrity.

The results are also in accordance with the reports of Amal *et al.*, (2015) who studied hepato-protective effect of ZnO NP in combination with Vitamin C in paracetamol induced oxidative stress in rats' hepatocytes. Silymarin is a natural compound derived from the species *Silybum marianum*, which is

commonly known as milk thistle. It is one of the medicinal plants used in treating illnesses, reported since ancestral times. Silymarin has been reported to have antioxidant, immunomodulatory, anti-fibrotic, anti-proliferative, and antiviral properties (Flora *et al.*, 1998).

The protective effect of SMN treatment was attributed to the presence of bioactive chemical components such as flavolignans, silydianin, silychristin, and silybin with antioxidant effects (Farghali *et al.*, 2000) which protect cellular membranes against oxidative damage (Nencini *et al.*, 2007).

The observations recorded in the present study were in accordance with those of several earlier workers (Jahovic *et al.*, 2003; Tugba *et al.*, 2010; Coleshowers *et al.*, 2010; Vardi *et al.*, 2010; Kose *et al.*, 2012; Tousson *et al.*, 2014; Calegari *et al.*, 2015; Deepak *et al.*, 2015; Abdelkar *et al.*, 2015, Vijaykumar *et al.*, 2016; Nema *et al.*, 2017; Nur *et al.*, 2018 and Cao *et al.*, 2019).

Comparison of ZnO NP treatment groups (Group IV and V) with silymarin treatment group (Group VI) it was observed that the hepato-protective effect of pre-treatment group was almost similar to that of silymarin treatment group. The results of the current study suggest that the ZnO NP pre-treatment is more effective in ameliorating MTX induced toxicity and the effect is equivalent to that of silymarin.

ZnO NPs can ameliorate considerably the toxic effects of MTX in rats. Concurrent treatment of ZnO NPs in comparison with pre-treatment is less effective. However, it ameliorates the toxic effects of MTX gradually with prolonged treatment (Fig. 1-4 and Table 1-4).

Table.1 Mean \pm SE values of malondialdehyde (MDA) levels (nmoles/mg of tissue) in rat liver of different groups at different time intervals

Groups	7 th day	21 st day	45 th day
Group I (Control)	0.923 \pm 0.012 ^{ax}	0.980 \pm 0.018 ^{ax}	1.00 \pm 0.024 ^{ax}
Group II (MTX)	5.708 \pm 0.344 ^{bx}	7.015 \pm 0.320 ^{by}	4.644 \pm 0.234 ^{bz}
Group III (ZnONP)	1.022 \pm 0.049 ^{ax}	0.987 \pm 0.020 ^{ax}	1.019 \pm 0.012 ^{ax}
Group IV (ZnONP+MTX)	3.920 \pm 0.168 ^{cx}	2.348 \pm 0.168 ^{cy}	1.762 \pm 0.080 ^{cz}
Group V (MTX+ZnONP)	5.042 \pm 0.240 ^{bx}	4.50 \pm 0.914 ^{dy}	2.892 \pm 0.085 ^{dz}
Group VI (MTX+SIL)	4.573 \pm 0.140 ^{cbx}	3.300 \pm 131 ^{ey}	1.963 \pm 0.206 ^{cz}

Values with different superscripts vary significantly at P<0.05

Table.2 Mean \pm SE values of superoxide dismutase (SOD) levels (U/min/mg protein) in rat liver of different groups at different time intervals

Groups	7 th day	21 st day	45 th day
Group I (Control)	31.872 \pm 1.625 ^{ax}	30.630 \pm 1.248 ^{ax}	32.317 \pm 0.989 ^{ax}
Group II (MTX)	8.845 \pm 0.614 ^{bx}	11.730 \pm 0.637 ^{bx}	17.112 \pm 1.136 ^{by}
Group III (ZnONP)	37.673 \pm 0.880 ^{cx}	42.753 \pm 1.213 ^{cy}	45.00 \pm 0.730 ^{cy}
Group IV (ZnONP+MTX)	23.442 \pm 1.219 ^{dx}	28.966 \pm 1.678 ^{ay}	37.194 \pm 0.825 ^{az}
Group V (MTX+ZnONP)	15.595 \pm 0.911 ^{ex}	27.00 \pm 0.756 ^{ay}	33.542 \pm 1.887 ^{az}
Group VI (MTX+SIL)	24.506 \pm 1.018 ^{dx}	31.543 \pm 2.022 ^{ay}	40.815 \pm 0.713 ^{caz}

Values with different superscripts vary significantly at P<0.05

Table.3 Mean \pm SE values of Catalase (CAT) levels (μ mol/min/mg protein) in rat liver of different groups at different time intervals

Groups	7 th day	21 st day	45 th day
Group I (Control)	47.18 \pm 0.418 ^{ax}	51.588 \pm 0.902 ^{ay}	51.612 \pm 0.448 ^{ay}
Group II (MTX)	18.845 \pm 0.180 ^{bx}	21.883 \pm 0.399 ^{by}	27.677 \pm 0.522 ^{bz}
Group III (ZnONP)	47.378 \pm 0.539 ^{ax}	50.622 \pm 0.629 ^{ay}	51.792 \pm 0.332 ^{ay}
Group IV (ZnONP+MTX)	26.371 \pm 1.484 ^{cdx}	33.462 \pm 1.488 ^{cy}	39.635 \pm 0.688 ^{cz}
Group V (MTX+ZnONP)	22.787 \pm 1.360 ^{cx}	29.358 \pm 0.693 ^{dy}	35.590 \pm 1.059 ^{dz}
Group VI (MTX+SIL)	29.805 \pm 1.448 ^{cdx}	37.152 \pm 0.996 ^{cy}	41.742 \pm 1.137 ^{cz}

Values with different superscripts vary significantly at P<0.05

Table.4 Mean ± SE values of glutathione peroxidase (GPx) levels (µmol/mg protein) in rat liver of different groups at different time intervals

Groups	7 th day	21 st day	45 th day
Group I (Control)	35.361 ± 1.918 ^{ax}	36.410 ± 1.957 ^{ax}	35.6880 ± 1.672 ^{ax}
Group II (MTX)	9.443 ± 0.239 ^{bx}	13.340 ± 18.768 ^{by}	13.340 ± 18.768 ^{bz}
Group III (ZnONP)	53.471 ± 0.848 ^{cx}	58.341 ± 0.338 ^{cy}	62.332 ± 0.185 ^{cz}
Group IV (ZnONP+MTX)	35.719 ± 1.720 ^{ax}	47.425 ± 1.704 ^{dy}	56.508 ± 0.452 ^{dz}
Group V (MTX+ZnONP)	26.513 ± 1.588 ^{dx}	34.360 ± 1.372 ^{ay}	41.160 ± 0.916 ^{az}
Group VI (MTX+SIL)	39.535 ± 2.077 ^{ax}	63.999 ± 1.920 ^{cy}	71.191 ± 1.408 ^{ez}

Values with different superscripts vary significantly at P<0.05

Fig.1 Mean ± SE values of malondialdehyde (MDA) levels (nmoles/mg of tissue) in rat liver of different groups at different time intervals

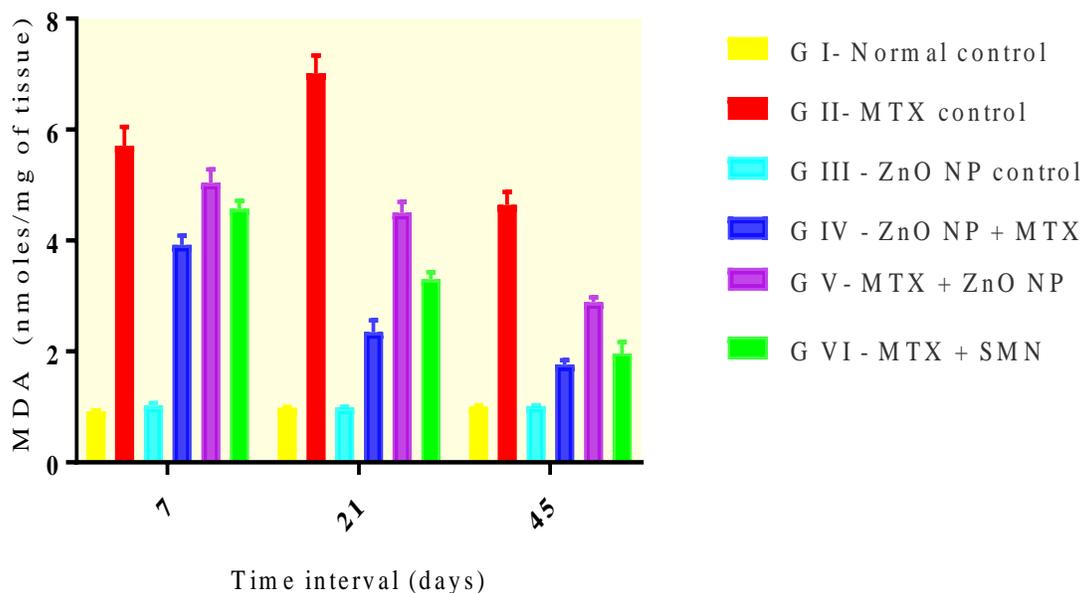


Fig.2 Mean \pm SE values of superoxide dismutase (SOD) levels (U/min/mg protein) in rat liver of different groups at different time intervals

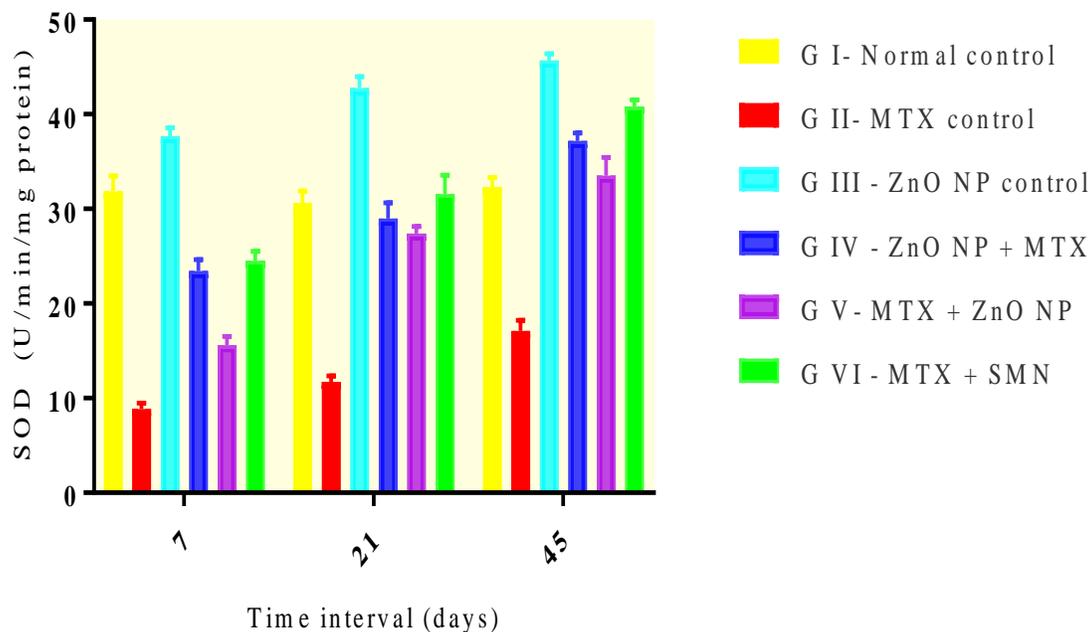


Fig.3 Mean \pm SE values of catalase (CAT) levels (μ moles/min/mg of protein) in rat liver of different groups at different time intervals

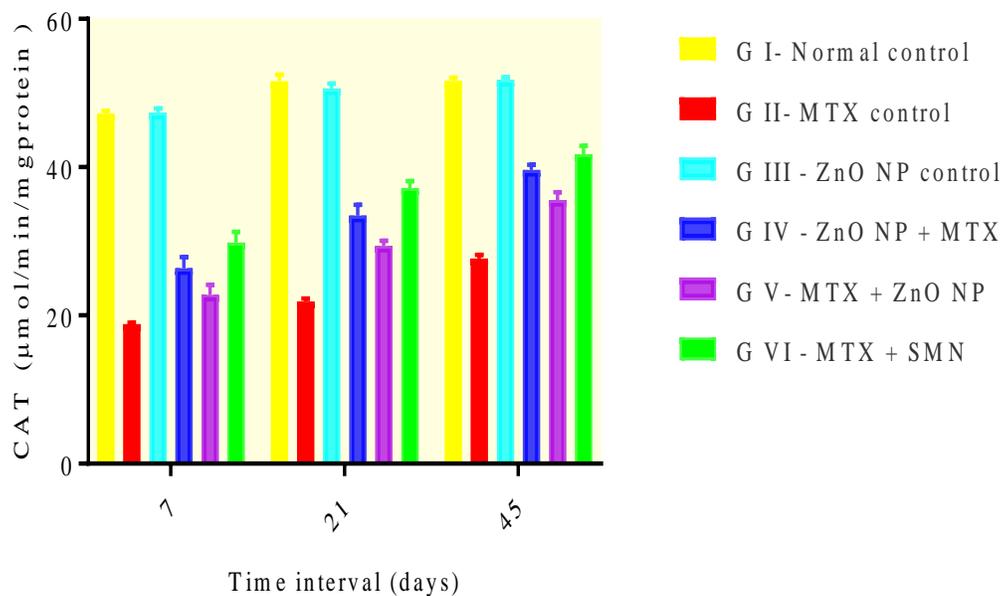
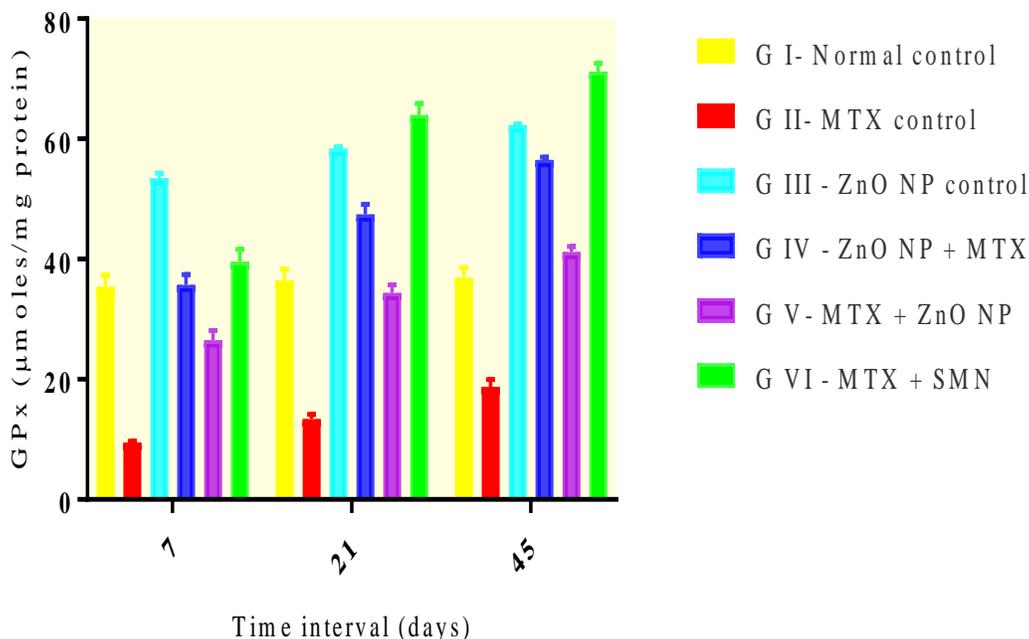


Fig.4 Mean \pm SE values of glutathione peroxidase (GPx) levels (μ moles/mg of protein) in rat liver of different groups at different time intervals



Pre-treatment of ZnO NPs has prophylactic effect and can effectively protect the animal from the immediate side effects of MTX therapy which is on par with the protective effect delivered by a proven herbal hepatoprotectant, silymarin. ZnO NP at 50 mg/kg b.w orally has antioxidant and anti-apoptotic activity and could be used as a prophylactic measure in combating drug induced immediate toxicities.

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