WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 4, 1445-1452. Research Article ISSN 2277– 7105

# ORAL DOSING OF METHYL PREDNISOLONE IN ADULT ZEBRAFISH AND EVALUATION OF HEPATO-PROTECTIVE ACTIVITY OF SUCCINATE DEHYDROGENASE AND ANTI-OXIDANT ACTIVITY OF GSX AND GST

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Article Received on
15 Feb 2016,
Revised on 06 March 2016,
Accepted on 26 March 2016
DOI: 10.20959/wjpr20164-5986

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## ABSTRACT

Methyl Prednisolone like most adrenocorticol steroids typically used for its anti-inflammatory effects. However, glucocorticoids have a wide range of effects, including changes to metabolism and immune responses. Common uses induces arthritis and short term treatment of bronchial inflammation or acute bronchitis due to various respiratory diseases. This study aims at use of Methyl Prednisolone and its effects on various organs and tissues of adult zebrafish and its related antioxidant enzymes like Glutathione Peroxide (GPx) and Glutathione-S-transperase and Succinate dehydrogenase enzyme. The LC 50 for Methyl Prednisolone was determined as 50ppm. As the concentration of drug dose was increased the activity of various enzymes was seen to increase exponentially. The activity of GPx was highest in muscles at both 20ppm and 10ppm followed by liver and gill had significant increase against control fishes. Similarly, the activity of GST and succinate dehydrogenase was highest in liver at both 20ppm and 10ppm followed by gill and muscle which had a significant increase against untreated zebrafish. The observation in this research reflects that all the enzymes activities were significantly enhanced in all the tissues (Gill, Muscle, liver)

when compared to Methyl prednisolone unexposed ones. This obtained result could be due detoxification mechanism exhibited by the *Danio rerio* on exposure to Methyl Prednisolone.

**KEYWORDS:** Methyl Prednisolone, *Danio rerio*, Glutathione Peroxide (GSx), Glutathione-S-transferase, Succinate dehydrogenase.

## INTRODUCTION

*Danio rerio* commonly known as Zebra fish belonging to the minnow family (Cyprinidae) of the order Cypriniformes. This fresh water tropical fish has five blue stripes on the sides of the body which extend to the caudal fins and resembles the stripes of a zebra fish, hence named as zebra fish.

Zebra fish has been extensively used in cloning, transgenesis and mapping approaches led to the use of zebra fish in higher eukaryotic development. Through genetic screening early development zebra fish mutants were identified in 1990s, established zebra fish as a main stream model for Human diseases. The development of transparent zebra fish variety, Casper has led to optical visualization o various cellular processes, embryonic developments, functioning of brain, certain diseases like cancer and leukemia.

The embryonic development of zebra fish is very rapid: In the first 24 hours after fertilization, all major organs form and within 3 days the fish hatch and start looking for food. After 3-4 months zebra fish are sexually mature and can generate new offspring. Large numbers of mutations that disrupt embryonic development have now been isolated in the zebra fish, many of which may serve as models for Human diseases. These mutants will help to understand the genetic networking controlling the development of vertebrates including Humans.

The zebra fish genome: Zebra fish have 25 chromosomes and their genome consists of about 1.5×10 billion basepairs against Human genome size of 3 x 10 billion base pairs.[1]

Zebra fish (*Danio rerio*) has a gained popularity as a pre-clinical screen in pharmaceutical research owing to its small size, large fecundity, rapid development, affordable maintenance and a complex vertebrae system conserved within its sequenced genome. Even though embryonic and larval stages of the fish are more popular in research, adult zebra fish are increasingly being reported as model organisms for studies on pharmacology[2] and neurosciences.[3] Conventionally studies in adult zebra fishes are conducted by dissolution of

chemicals to the aquarium water at different concentrations and assessing their effects, which in turn requires huge doses of drugs in order to meet therapeutic concentrations and the actual dosage of the drug in terms of milligrams per kilogram(mg/kg) of body weight cannot be considered as drug exposure is via multiple bodily routes.

Besides attracting ethical concern, the use of mammals is expensive and labour intensive, limiting an application in large-scale screening programs. Embryos and larvae of the lower vertebrae zebrafish (*Danio rerio*) may offer a cheap and effective alternative.[4] Zebrafish embryos/larvae are small and can be obtained in large numbers throughout the year at a fraction of the cost of a mouse or a rat. Zebrafish embryos develop outside of the mother allowing systematic compound screens.

## MATERIALS AND METHODS

### *Aquaculture*

All procedures for experimentation were carried out as per Guidelines for Use of Zebrafish in the NIH Intramural Research Program (http://oacu.od.nih.gov/ARAC/documents/Zebrafish.pdf) and the Zebrafish book[5] Experimental fishes were bought from local pet shop, Bengaluru, India. Zebra fish (*Danio rerio* sp.) which is used as a object for sub lethal test, has approximately 3.1-4.6cm in length and their average weight is about 0.295 to 0.847g. Zebra fish were then housed separately by gender under a 14 hrs/ 10hrs dark cycle photo period (ie. 9am to 11 pm light).

Prior to experimentation fishes were allowed to acclimatize to laboratory conditions for a month. Fish were maintained in a recirculation system with polysulphone housing tanks containing purified water (Millipore ELIX sytem grade) with 0.2% sea salt at $28^0$C under a 14:10 h light and dark cycle. Fish were fed three times daily with live hatched brine shrimp and dry food. Zebrafishes were fasted overnight and weighed prior to the study. On the day of dosing, fresh Methyl Prednisolone in 1% DMSO was administered orally at the required dose with the dose volume of 10µl/kg body weight of zebrafish. The dose volume was rounded-off to the nearest single decimal of the microlitre.

In the present study, was administered at a rate of 5mg/kg body weight, this method of oral administration involves the use of a micropipette with a small that is gently inserted into a mouth and pharynx of zebrafish.[6] The test drug solution/suspension is then gently released into the fish ensuring that the administered solution does not regurgigate. During training

personnel, permitted food grade colored solution were used to ensure no spillage occurs either through oral cavity or gill filaments.

Methyl Prednisolone was purchased from CDH, Bengaluru, India and was dissolved in 1% DMSO to form stock solution and stored at room temperature. 10 fishes were randomly selected from the stock and exposed to different concentrations of Methyl Prednisolone (10,20,30,40,50,60,70,80,90 and 100 ppm) for 96 hrs to determine the median lethal concentration (LC 50) of CBZ with selection exposure of 15 ppm for chronic sub-lethal concentration exposure studies. The LC 50 value for Methyl Prednisolone was 40ppm. For further sub-lethal study, 10 ppm and 20 ppm were chosen. A control group was maintained simultaneously. All these experiments were performed in triplicates.

***Sample preparation***

Tissue homogenate preparation the gill, muscle and liver of the fishes from the exposed and non exposed groups were dissected carefully and weighed for biochemical analysis, after homogenized to 1/10 (w/v) ratio of cold physiological saline solution Nacl (0.86%) using a mortar and pestle and then, centrifuged at 8000 r/min in $4^0$C, the supernatant was used for biochemical analysis. Succinate dehydrogenase activity was determined by method of[7] Total protein content was quantified by the procedure of reference[9] at 595 nm and using bovine serum albumin as standard.

***Statistical analysis***

Results of the experiment were expressed as mean and standard error of mean of different groups. The differences between the mean values were evaluated by ANOVA (16.0). The values for $P< 0.001$ were considered significant. Accordingly, a Statistical Software Package (SPSS) was used.

**RESULTS AND DISCUSSION**

The enzyme activity of various tissues were assayed in exposed and non exposed groups of zebrafishes exposed to Methy prednisolone. The data shown in Table -1 indicates that MPN at sublethal concentrations (10 ppm: 5.161 ± 0.032 U/mg protein; 20 ppm: 7.140 ± 0.024 U/mg protein) have significantly increased ($F = 4.55E3$, $P < 0.001$) GPx activity of gill when analysed against control (3.60 ± 0.032 U/ mg protein). Similarly significant increase $F= 4.25E4$, $P<0.001$) in GPx activity of liver was increased (10 ppm: 6.248 ± 0.031 U/mg protein; 20 ppm: 10.310 ± 0.030 U /mg protein) when compared to the MPN unexposed ones

(7.121 ± 0.012 U/mg protein). Muscle GPx significantly (F=2.291E4) increased in Zebrafishes exposed to 10 ppm and 20 ppm (9.112 ± 0.100 U/mg protein; 12.240 ± 0.031 U/mg protein respectively) when compared to the control fishes (8.950 ± 0.021 U/mg protein). Using the available data, dose dependent relationship was seen between methyl prednisolone concentration and GPx activity of Gill, Liver and Muscle.

**Table-1 Changes in the levels of Glutathione Peroxide (GPx) of the various tissues of *Danio rerio* exposed to Methyl Prednisolone.**

| MPN Treatment | Gill U/mg protein | Liver U/mg protein | Muscle U/mg protein |
|---|---|---|---|
| Control | $3.60 \pm 0.032^{\Psi}$ | $7.121 \pm 0.021^{\Psi}$ | $8.950 \pm 0.021^{\Psi}$ |
| 10 ppm | $5.161 \pm 0.030^{\beta}$ | $6.248 \pm 0.031^{\beta}$ | $9.112 \pm 0.100^{\beta}$ |
| 20 ppm | $7.140 \pm 0.024^{\alpha}$ | $10.310 \pm 0.030^{\alpha}$ | $12.240 \pm 0.031^{\alpha}$ |
| F- value | 4.559E3 | 4.251E4 | 2.291E4 |

***** Signifiacnt at P < 0.001. In a coloumn, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT).**

Methyl prednisolone also had its significant effect on the activity of GST of gill (10 ppm: 14.310 ± 0.020 U/mg protein; 20 ppm 17.348 ± 0.020 U/mg protein) (F=6.210E3, P<0.001). Whereas, MPN untreated fish showed gill GST activity of 13.145 ± 0.059 U/mg protein and MPN exposure on liver tissue showed at (16.243 ± 0.021 U/mg protein and 27.910 ± 0.0351 U/ mg protein, 10 ppm and 20 ppm respectively). Significantly (F= 1.568E3, P< 0.001) higher. Liver GST activity was registered when compared to the control (7.211 ± 0.041 U/mg protein). From table-2 it is evident that the muscle GST activity of MPN exposed zebrafishes showed significant increased (F= 4.079E3, P< 0.001) with 10 ppm and 20 ppm values of 6.643 ± 0.020 and 8.193 ± 0.028 U/mg protein respectively when compared against the control 5.159 ± 0.031 U/mg protein). In lines with GPx, GST activity also showed dose dependent relationship with MPN concentrations.

**Table- 2 Changes in the levels of Glutathione-S-transferase (GST) of the various tissues of *Danio rerio* exposed to Methyl Prednisolone.**

| MPN Treatment | Gill U/mg protein | Liver U/mg protein | Muscle U/mg protein |
|---|---|---|---|
| Control | $13.145 \pm 0.061^{\Psi}$ | $7.211 \pm 0.041^{\Psi}$ | $5.159 \pm 0.031^{\Psi}$ |
| 10 ppm | $14.310 \pm 0.020^{\beta}$ | $16.243 \pm 0.021^{\beta}$ | $6.643 \pm 0.020^{\beta}$ |
| 20 ppm | $17.348 \pm 0.020^{\alpha}$ | $24.710 \pm 0.031^{\alpha}$ | $8.193 \pm 0.028^{\alpha}$ |
| F- value | 6.210E3 | 1.568E3 | 4.079E3 |

***** Signifiacnt at P < 0.001. In a coloumn, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT).**

Significant increase (F= 176.1E3, P< 0.001 ) in Succinate dehydrogenase activity of gill, was observed in zebrafishes when exposed to mpn (10 ppm: 15.106 ± 0.049 U/mg protein; 20 ppm: 16.661 ± 0.069 U/mg protein) when compared against control (13.206 ± 0.061 U/mg protein). Liver also had shown response to MPN by producing increased Succinate dehydrogenase activity. As the MPN concentration increased, liver succinate dehydrogenase activity also gets elevated (10 ppm: 16.106 ± 0.049 U/mg protein; 20 ppm: 21.764 ± 0.014 U/mg protein), whereas, controlled showed succinate dehydrogenase liver activity of 15.153 ± 0.038 which were found significantly lower (F=6.753E3, P<0.001) when compared to the MPN treated ones. MPN significantly elevates (F=1.78E4, P<0.001) muscle succinate dehydrogenase activity on zebrafishes when compared to the control fishes (12.193 ± 0.098 U/mg protein). Carbamazepine at 10 ppm and 20 ppm showed muscle AChe activity of 16.281 ± 0.011 U/mg protein and 15.266 ± 0.012 U/mg protein, respectively as shown in table-3.

**Table-3 Changes in the levels of Succinate dehydrogenase of the various tissues of *Danio rerio* exposed to carbamazepine.**

| MPN Treatment | Gill U/mg protein | Liver U/mg protein | Muscle U/mg protein |
|---|---|---|---|
| Control | $13.206 \pm 0.061^{\Psi}$ | $15.153 \pm 0.038^{\Psi}$ | $12.193 \pm 0.078^{\Psi}$ |
| 10 ppm | $15.106 \pm 0.049^{\beta}$ | $16.106 \pm 0.049^{\beta}$ | $16.281 \pm 0.011^{\beta}$ |
| 20 ppm | $16.661 \pm 0.069^{\alpha}$ | $21.764 \pm 0.014^{\alpha}$ | $15.266 \pm 0.012^{\alpha}$ |
| F- value | 176.1E3 | 6.753E4 | 1.78E4 |

***** Signifiacnt at P < 0.001. In a coloumn, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT).**

## CONCLUSION

The present experiment involves oral drug administration in adult zebrafish using an accurate method that is simple, non-invasive, non-traumatic and can ensure precise delivery of most drugs and formulations through the oral route. These reports suggest that zebrafish may be an excellent experimental model organism for studying toxicity levels and expression of antioxidant enzymatic genes like GPx, GST and enzyme involved in energy production succinate dehydrogenase.

The present study correlates to the study carried out by Jayanth *et al*., (2014) who have demonstrated that activities of anti-oxidative enzymes was significantly (P< 0.01) influenced by rotenone in dose dependent manner in *Danio rerio*. They have seen significant (P <0.01) rise in the activity of GPx, GST, Succinate dehydrogenase in *D.rerio*

Glutathione peroxide is an antioxidant enzyme involved in the elimination of ROS (Reactive Oxygen Species). The antioxidant enzymes that make up the antioxidant defence system are expected to be intrinsically linked and dependent upon the activity of one another, therefore, one can expect to see correlative changes in the activity of GST also.

Succinate dehydrogenase activity was highest in liver, followed by gill and muscles at both 10and 20 ppm concentrations of methyl prednsisolone.[10]

**ACKNOWLEDGEMENTS**

Authors are also grateful to authors/editors/ publishers of all those articles, journals and books from where the literature of this article has been reviewed and discussed. The authors are also grateful to Dr. Guru Prasad Ramakrishna, Director, Durga Femtotechnologies and Research Scientist, Bengaluru, India and Dr. B.P. Harini for constant support and encouragement.

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