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Comparative analysis based on 2-methanesulfonylpyridine (MSP) in blood plasma of fresh water fish under the exposure of toxicants

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ABSTRACT

Present investigation was focused on evaluation of hair care products containing ZnPT only and ZnPT with EDTA. Toxicity comparison was based on MSP content in fresh water fish plasma under the exposure of shampoos containing ZnPT alone and a combination of ZnPT and EDTA. Blood samples were collected before treatment and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24 hrs after treatment. Plasma MSP concentrations were not measurable from 0.5 hrs 8 hrs after treatment with shampoo containing 1% ZnPT, and from 0.5 to 8 hrs after treatment with shampoo containing 1% ZnPT + 2% EDTA. Thereafter, MSP concentration kept elevating up to 24 hr in both groups. This composition is speculated to increase ZnPT absorption and the resulting increased toxicity in aquatic organisms.

Keywords: EDTA, Fresh water fish, Zinc Pyrithione, EDTA, Metabolite

1. INTRODUCTION

The broad-spectrum antimicrobial zinc pyrithione (ZnPT) is widely used as preservative in paints to antidandruff shampoo. ZnPT is used as active antimicrobial agents in antidandruff shampoos. ZnPT mainly inhibit the development of microorganisms, especially specific fungus species, on the human scalp. Apart from this, Zinc pyrithione (ZnPT) is also widely used metal pyrithione booster biocides in many copper-based antifouling paints (Konstantinou and Albanis, 2004). In recent decades, the use of TBT has been strictly banned due to its persistence and toxic effects on non-target aquatic organisms. Since the global ban of TBT, ZnPT has been globally accepted as a substitute (Konstantinou and Albanis, 2004).

Although, hair care institutes do not release the rinse water containing shampoo into any water source directly but in India people use hair care products during the holy bath (a mythological ritual) in various fresh water streams. Due to this practice the rinse hair water containing ZnPT gets mixed in river. It has been reported by previous investigators that ZnPT is readily photo-degraded under direct sunlight to less toxic compounds in the aquatic environment as half-life is less than 2 mins (Turley *et al.*, 2000). It was further suggested that in water columns with high turbidity, ZnPT may accumulate in the sediment (Maraldo and Dahllöf, 2004). Marcheselli *et al.*, (2010) found that half of the initial ZnPT quantity remained after 48-h light exposure (indirect sunlight). A total pyrithione concentration of 105 nM was detected in water samples, indicating the possible existence of pyrithiones like ZnPT and CuPT in marine water columns (Mackie et al., 2004) and Marcheselli *et al.* (2010b).

EDTA is useful to improve stability in shampoo formulations and in current situation the formulation of ZnPT and EDTA is widely used worldwide. This composition is speculated to increase ZnPT absorption by EDTA, and the resulting toxicity is concerning (Mochida, *et al.*, 2009). ZnPT metabolized into its metabolites mainly 2-methanesulfonylpyridine (MSP) $C^6H^7NO^2S$. Among the

metabolites, 2-methanesulfonylpyridine (MSP) has been identified as a major serum metabolite of ZnPT (Mochida, *et al.*, 2009).

This investigation was focused to evaluate the effect of EDTA on ZnPT exposure by measuring MSP content in fish plasma under the exposure of shampoos containing ZnPT alone and a combination of ZnPT and EDTA.

2. MATERIALS AND METHODS

Reagents: Zinc pyrithione, EDTA, Imipramine HCl and d3-5-Nitro-5'-hydroxy-indirubin-3'-oxime were purchased from HIMEDIA (about 95% India). All other chemicals and reagents were of analytical grade and used without further purification.

Preparation of dose: Shampoo formulations were prepared by adding ZnPT alone and ZnPT and EDTA in a commercial shampoo product (a widely used brand among population, Sunsilk, India) to yield a final concentration of 5% ZnPT or 5% ZnPT + 10% EDTA, respectively (Jung, *et al.,* 2019). The shampoo formulations were mixed thoroughly and then diluted 5 times with distilled water. The final diluted shampoo formulations (1% ZnPT or 1% ZnPT + 2% EDTA) were used in experiments.

Test organisms: Samples of *Puntius conchonius* were collected from Ganga river with the help of local support. Mean Total Length (TL) of samples was recorded 5.57 ± 1.23 cm and mean weight (MW) was 16.68 ± 3.72 g. Samples were kept in plastic tanks and tanks were cleaned at alternate day during acclimatization period and water was changed every 48-hour. Fishes were kept at room temperature with 12h:12h (light:dark) photoperiod and fed with wheat flour balls for 1 week before treatment. Fishes were fasted for 12 hr prior to the start of dosing.

The final diluted shampoo formulations (1% ZnPT and 1% ZnPT + 2% EDTA) were used in experiments. The animals were then assigned to two groups. Group (A) was treated with shampoo containing 1% ZnPT, whereas group (B) was treated with shampoo containing 1% ZnPT + 2% EDTA (Jung, *et al.,* 2019). Blood samples were collected before treatment and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24 hrs after treatment. After exposure, three samples at each time interval were caught with a dip net and stunned with a blow on the head. The caudal artery at the peduncle was punctured. Blood samples (approximately 0.2 ml) were collected in sampling tubes by using syringes. Blood samples were taken within 40 seconds after neutralization of the fish. Immediately after collection, blood samples were centrifuged for 1-2 min at 10,000-13,000 rpm. The obtained plasma samples were stored at 0^{o}C until analysis (Jung, *et al.*,2019).

Internal standard solution (120 µL; imipramine) was added to plasma and the mixture was vortex-mixed thoroughly for 30 sec. After centrifugation at 4^{o}C and 13,000 rpm for 10 min, the supernatants were diluted with 2 times volume of distilled water (Jung, *et al.,* 2019). All chromatographic peaks were reviewed and calibration curves were prepared using a linear regression. All data were expressed as mean ± SD.

3. RESULTS

MSP concentration in plasma after treatment with shampoo containing 1% ZnPT or 1% ZnPT + 2% EDTA are presented in Table 1. Statistical analysis was performed with the help of SPSS statistical software package (ver. 15.0) and Box plot were made by ALCULA software. Maximum percentage (118%) difference of MSP concentrations between both combinations was recorded at 12 hrs of sample after treatment whereas minimum percentage (69%) difference of MSP concentrations between both combinations was recorded at 20 hrs.

DISCUSSION

This study was focused to analyze the effect of EDTA on ZnPT toxicity. Analysis of results showed significant fluctuations in MSP concentrations at regular time intervals between the two combinations of shampoo, containing 1% ZnPT only and 1% ZnPT + 2% EDTA. MSP concentration, was approximately 118% (max.) and 69% (Min.) higher in the 1% ZnPT + 2% EDTA shampoo group than in the 1% ZnPT shampoo group in 24 hr blood sample.

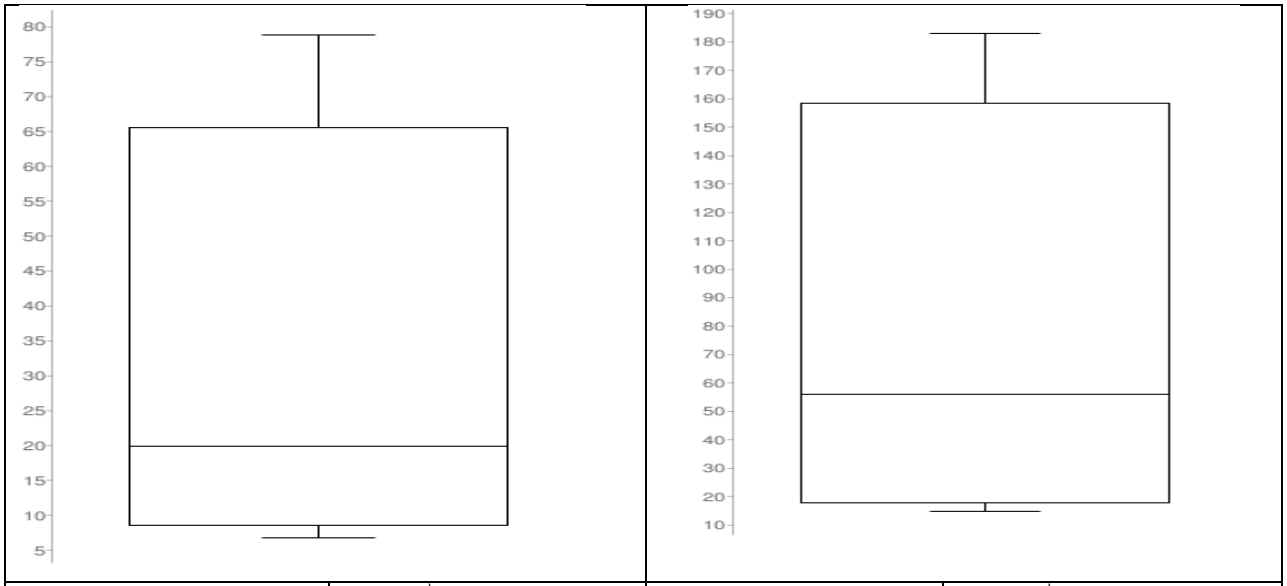
Compared to plasma MSP concentrations were found absent in 1% ZnPT group from 0.5 hr to 8 hrs of exposure and in 1% ZnPT + 2% EDTA combination at 0.5 hr to 8 hrs of dosing. However, first recognizable concentration of MSP was recorded at 12th hr of exposure in 1% ZnPT + 2% EDTA shampoo group whereas in 1% ZnPT group it was recorded at 12th hrs of blood sample. Three minor serum metabolites of ZnPT, 2-(methylthio)pyridine-1-oxide, 2- (methylthio)pyridine and 2-(methylsulfinyl) pyridine-1-oxide, are the intermediate compounds in the biotransformation of ZnPT to MSP (Kobayashi and Okamura, 2002). MSP has been identified as a major serum metabolite of ZnPT because MSP is a very stable and abundant metabolite of ZnPT and positioned at the final step of the metabolic pathway of ZnPT, therefore it can be used as a good indicator of systemic ZnPT exposure. In both groups, plasma MSP concentration gradually increased with time and reached a maximum concentration at 12 hr. There were significant differences in MSP concentration between the two groups. Gibson, *et al.*, (1982) and Jung, *et al.*, (2019) reported that MSP in the circulation increased with time after ZnPT dosing in organisms and it was the only prominent circulating metabolite at the later time points.

Table. 1. Plasma MSP concentrations (ng/mL with mean ±SD) of *Puntius conchonius*

	(A)	(B)	(C)	Difference between (B) and (C) in %
Time Interval (Hours)	Control Group	1% ZnPT (ng/mL)	1% ZnPT + 2% EDTA (ng/mL)	
0.5	Absent	Absent	Absent	CBD
1	Absent	Absent	Absent	CBD
2	Absent	Absent	Absent	CBD
3	Absent	Absent	Absent	CBD
4	Absent	Absent	Absent	CBD
6	Absent	Absent	Absent	CBD
8	Absent	Absent	Absent	CBD
12	Absent	6.83±2.7	14.9± 4.8	118%
16	Absent	13.8±3.1	27.3± 9.4	97%
20	Absent	26.1±6.2	84.6± 12.3	69%
24	Absent	78.8±16.4	182.9±19.8	78%

(CBD= Cannot Be Determined)

Figure 2. Box plot of MSP concentrations in plasma of *Puntius conchonius*



Median: 19.95	3rd quartile:65.625	Median: 55.95	3rd quartile:158.325
1st quartile:8.572	Inetrquartile:57.0525	1st quartile:18.000	Inetrquartile:140.325
1% ZnPT combination		**1% ZnPT + 2% EDTA combination**	

4. CONCLUSIONS

In the light of data recorded during study, it may be stated that toxicity of ZnPT increases when combined with EDTA. The rinse-off hair water containing 1% ZnPT and EDTA when mixed in river water during the holy bath of devotee (due to the use of antidandruff shampoo). 1% ZnPT + EDTA remains for longer duration and found more toxic than containing 1%ZnPT alone formula. Therefore, this study recommends avoiding such hair products during holy bath or near any natural water source. Further extensive studies are needed on other aquatic organism to evaluate the adverse effect of EDTA on exposure to ZnPT.

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