ijlpr 2021; doi 10.22376/ijpbs/lpr.2021.11.6.L130-136

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Monocrotophos Toxicity

Toxic Effect Of Monocrotophos On Various Blood Parameters In Edible Freshwater Fish *Oreochromis mossambicus (Tilapia)*

A.Solaiappan [1]* and J.Prakash Sahaya Leon [2]

[1]Department of Zoology, Government Arts College for Men, Krishnagiri-1, Tamilnadu, India, Affiliated to Periyar University, Salem

[2]Department of Zoology, Government Arts College for Men, Krishnagiri-1, and Tamilnadu, India, Affiliated to Periyar University, Salem

Abstract : In modern agriculture, farmers are using the authenticity of pesticides for agricultural activities. Water bodies are often polluted with various harmfull chemical substances. Fishes are very important for human nourishment. In this study, the effect of Pesticide monocrotophos on various hematological parameters such as RBC, WBC, and Hemoglobin was observed in freshwater fish *Oreochromis mossambicus*. The fish were exposed to various sub-lethal concentrations (1/10, 1/20, and 1/30) of Monocrotophos for 10, 20, and 30 days. The blood samples were analyzed at the end of every exposure period. Increased count of WBC observed in Lower Sub lethal concentration (21.25, 21.76, 22.01), Medium Sub-lethal concentration (21.69, 22.48, 22.89), and Higher Sub lethal concentration (22.47, 23.54, 23.69) of monocrotophos at various monocrotophos exposure periods on experimental fish *Oreochromis mossambicus*. Decreased count of RBC observed in Lower Sub-lethal concentration (3.58,3.21,3.08), Medium Sub-lethal concentration (2.97,2.35,2.19) and Higher Sub-lethal concentration (2.41,2.16,2.02) and decreased level of Hemoglobin were observed in Lower Sub-lethal concentration (21.25,21.76,22.01), Medium Sub-lethal concentration (21.69,22.48,22.89) and Higher Sub-lethal concentration (22.47,23.54,23.69) of monocrotophos at various exposure period on fish *Oreochromis mossambicus*. The 1/10 sub-lethal concentration of monocrotophos showed a high level of variation on 30th-day exposure. Reduction of RBC indicates hemolysis in fish and increased WBC count has been considered to be an adaptation of animals to meet stressful conditions. In the present study, it was found that the effect of monocrotophos alters the activity of various blood parameters in freshwater fish *Oreochromis mossambicus.* Therefore, the experiment is an attempt to study the toxicity of the pesticide Monocrotophos concerning blood parameters of freshwater fish *Oreochromis mossambicus.* It is concluded from this study that exposure to Monocrotophos affects the hematological parameters of fish.

Keywords: *Oreochromis mossambicus*, Hemoglobin, White Blood Cells (WBC), Red Blood Cells (RBC), Monocrotophos, edible fish

***Corresponding Author**

A.Solaiappan , Department of Zoology, Government Arts College for Men, Krishnagiri-1, Tamilnadu, India, Affiliated to Periyar University, Salem

Received On 24 June, 2021
Revised On 22 November, 2021
Accepted On 27 November, 2021
Published On 30 November, 2021

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation A. Solaiappan and J.Prakash Sahaya Leon , Toxic Effect of Monocrotophos on Various Blood Parameters in Edible Freshwater Fish Oreochromis Mossambicus (Tilapia).(2021).Int. J. Life Sci. Pharma Res.11(6), L130-136
http://dx.doi.org/10.22376/ijpbs/lpr.2021.11.6.L130-136

1. INTRODUCTION

Insect repellent like pyrethroids are considered the most harmful for aquatic life[1]. Pesticides, applied in some restricted areas also were carried away by rains to water bodies like rivers, ponds and alters the physicochemical properties of water. Pesticides chemical properties remain unchanged or active for longer days in the environment, concentrating in the bodies of animals and threatening aquatic life[3]. Currently, pesticides are widely used in modern agriculture to aid in the production of pest-free food. However, some pesticides have the potential to cause serious health and environmental damage. The pesticide residues reach the environment by the runoff from agricultural fields and affect the biotic life[4]. Pyrethroid Pesticides are used mainly to control the pest [5]. The increasing use of pesticides mostly affects live organisms causing chemical pollution[6]. Farmers use monocrotophos in their fields for controlling insect pests. Residual of these pesticides create alteration into the ecosystem and disturb the healthy environment and aquatic forms. Monocrotophos is mainly used in agriculture and animal husbandry[7]. Non-targeted organisms like fish are mainly affected by those pesticides poisoning, which is also harmful for human consumption. Many harmful pesticides have been studied to produce several biochemical and hematological changes in freshwater fish at sub-lethal levels. Monocrotophos is one of the most preferable organophosphate pesticides, used by farmers. Pesticides change the blood parameters like RBC and WBC count and also change the level of Hb. Blood is the most important body fluid, acting as a vehicle for mobilizing defense mechanisms against diseases. Its composition reflects the physiological condition of the fish . Various tissues and organs of the body directly come in contact with blood. The physiological status of an organism is reflected in its blood parameters. Pesticides bind with blood proteins and induce changes in blood hematology like blood glucose level, serum protein levels, count of RBC, and WBC. The hematological study is an easy tool to determine the health of fishes[8]. Pathophysiological changes in animals were determined by Haematological parameters. Blood indices vary with different environmental conditions and chemicals[9]. Undesirable changes in the aquatic media reflect in the blood parameters through gills[10]. Blood components like RBC, WBC, and Hb are important diagnosing factors for identifying the functional status of the fish exposed to monocrotophos toxicants. Blood parameters can serve as a valuable tool in detecting biological and physiological changes taking place in aquatic animals[11]. Hematological parameters are used as an index to protect the fish species during stress conditions[12]. Hematological studies are frequently applied for the diagnosis of diseases in aquatic organisms like fish in the field of aquaculture[13]. The present study aims to analyze the effect of Monocrotophos with different sub-lethal (1/10, 1/20, 1/30) concentration on Hematological parameters (RBC, WBC, and Hb) of freshwater fish *Oreochromis mossambicus* exposed on different exposure periods (10, 20 and 30 days). The present study is vital because it analyses the health of fish, and also it monitors the long effects of pesticides on aquatic life.

2. MATERIALS AND METHODS

The freshwater fish *O.mossambicus* was procured from the KRP dam located in the Krishnagiri district, Tamil nadu , India. The fishes was authenticated by a zoologist at the department of Zoology, Government Arts College for Men, Krishnagiri-1, and Tamilnadu, India . The collected fishes were aggregated in a large cement tank for 4 weeks which was cleaned with 0.1% of the Potassium Permanganate solution. The fish were fed with a mixture of rice bran and groundnut oil cake. The average length of fish was 15 -17 cm and weight of 18 to 20 grams were selected for the study. Pesticide, Monocrotophos (25% EC) was used for the experiment. Finney's probit analysis method was used for finding the sub-lethal conc. of Monocrotophos[14]. The 96hr LC_{50} value of Monocrotophos was found as 4.9 mg/l. The experimental setup was made into four groups, Group-1 consist of 10 fish with pesticide-free water, Group-2 consist of 10 fishes with $1/10^{th}$ sub-lethal of cypermethrin treated water, Group-3 consisted of 10 Fishes with $1/20^{th}$ sub lethal of cypermethrin treated water, and Group-4 consist of 10 fish with $1/30^{th}$ sub lethal of cypermethrin treated water. At the end of every experimental period (10, 20, and 30 days) the Blood samples were collected from fishes of each experimental group (Eppendroff tubes with EDTA anticoagulant) [15]. Neubauer hemocytometer is used for the total count of RBC, WBC, and Sahli's method used for Hb Estimation[16].

3. STATISTICAL ANALYSIS

The values are expressed as mean ± SD. Data were statistically analyzed using SPSS 20.0 software by Analysis of Variance (ANOVA) along with Duncan's Multiple Range Test (DMRT)[17], which was applied to find out the significant difference between various treatment means and control means for the observed parameters. A probability value (P) of less than 0.05 was considered statistically significant.

4. RESULT

Fish, treated to various sub-lethal concentrations ($1/10^{th}$, $1/20^{th}$, and $1/30^{th}$) of Monocrotophos, showed a decreased level of RBC, WBC, and increased level of hemoglobin at the end of the 30^{th} day of the exposure period compared with the control group.

4.1 RBC COUNT

Fish, exposed at $1/10^{th}$, $1/20^{th}$, and $1/30^{th}$ sub-lethal concentration levels of Monocrotophos for 10, 20, and 30 days, showed decreased levels of RBC count compared with control. Maximum decreased level of RBC count observed in 30 days at $1/10^{th}$ of sub-lethal concentration of Monocrotophos treated experiment. The low value of RBC was observed at 10 days of exposure in $1/30^{th}$ level of sub-lethal concentration. (Table-1).

4.2 WBC COUNT

The total count of WBC was increased in Monocrotophos treated fish in all the exposure periods of 10, 20, and 30 days in various sub-lethal concentrations (1/10, 1/20, 1/30) compared to the control group. On the 30^{th} day, Monocrotophos exposed fish showed a maximum increased level of WBC count at $1/10^{th}$ sub-lethal concentration. The 10^{th} and 20^{th} day exposed fishes at 1/10, 1/20 and 1/30 sub-lethal concentration also showed increased levels of WBC count compared with the control group. (Table-2).

4.3 HAEMOGLOBIN PERCENTAGE

Decreased content of Hemoglobin was observed in all three exposure periods (10, 20, and 30 days) at various sub-lethal concentrations of (1/10, 1/20, and 1/30) monocrotophos compared with the control group. A highly decreased level of

Hb was observed in the 30 th day exposure period at 1/10th sub-lethal concentration of cypermethrin exposure. The 10 th and 20th day exposed fishes at 1/10, 1/20 and 1/30 sub-lethal concentrations of cypermethrin also showed decreased levels of Hemoglobin compared with the control group. (Table-3).

Table 1. RBC count (cu.mm) in the blood sample of *O.mossambicus* exposed to various sublethal concentration of Monocrotophos at various days of exposure.

GROUP	10 DAYS Exposure	20 DAYS Exposure	30 DAYS Exposure
Control	4.64±0.62[a]	4.86±0.52[a]	4.95±0.42[a]
Lower Sublethal concentration (1/30)	3.58±0.25[b]	3.21±0.35[b]	3.08±0.56[b]
Medium Sublethal concentration (1/20)	2.97±0.15[c]	2.35±0.41[c]	2.19±0.23[c]
Higher Sublethal concentration (1/10)	2.41±0.52[d]	2.16±0.57[d]	2.02±0.71[d]

Values are mean ± S.D., Sample Size (N) = 6. Values that do not share common superscript within row and column with superscripts a,b,c,d differ significantly at 5% (P<0.05) level between exposure and control groups.

Decreased level of RBC count was observed in a blood sample of experimental fish *Oreochromis mossambicus* in the various sub-lethal concentration of cypermethrin (1/10, 1/20, and 1/30) for 30 days when it is compared with a control group. A low count of RBC was observed in the higher sub lethal concentration at 30 days of exposure.

Table 2. WBC count (cu.mm) in the blood sample of *O.mossambicus* exposed to various sublethal concentration of Monocrotophos at various days (10,20 and 30) of exposure.

GROUP	10 DAYS Exposure	20 DAYS Exposure	30 DAYS Exposure
Control	20.29±0.05[a]	20.26±0.03[a]	20.27±0.07[a]
Lower Sublethal concentration (1/30)	21.25±0.32[b]	21.76±0.13[b]	22.01±0.11[b]
Medium Sublethal concentration (1/20)	21.69±0.25[c]	22.48±0.32[c]	22.89±0.36[c]
Higher Sublethal concentration (1/10)	22.47±0.32[d]	23.54±0.71[d]	23.69±0.29[d]

Values are mean ± S.D., Sample Size (N) = 6. Values that do not share common superscript within row and column with superscripts a,b,c,d differ significantly at 5% (P<0.05) level between exposure and control groups.

Increased level of WBC count was observed in a blood sample of experimental fish *Oreochromis mossambicus* in the various sub-lethal concentration of cypermethrin (1/10, 1/20, and 1/30) for 30 days when it is compared with a control group. A high count of WBC was observed in the higher sub-lethal concentration at 30 days of exposure.

Table 3. Hemoglobin (%) level in the blood sample of *O.mossambicus* exposed to various sublethal concentration of Monocrotophos at various days (10,20 and 30) of exposure.

GROUP	10 DAYS Exposure	20 DAYS Exposure	30 DAYS Exposure
Control	10.95±0.27[a]	10.91±0.13[a]	10.89±0.18[a]
Lower Sublethal concentration (1/30)	9.86±0.15[b]	9.52±0.34[b]	9.13±0.32[b]
Medium Sublethal concentration (1/20)	9.12±0.42[c]	9.01±0.19[c]	8.72±0.41[c]
Higher Sublethal concentration (1/10)	8.72±0.58[d]	8.21±0.35[d]	8.02±0.56[d]

Values are mean ± S.D., Sample Size (N) = 6. Values that do not share common superscript within row and column with superscripts a,b,c,d differ significantly at 5% (P<0.05) level between exposure and control groups.

Decreased content of Hb was observed in a blood sample of experimental fish *Oreochromis mossambicus* in the various sub-lethal concentration of cypermethrin (1/10, 1/20, and 1/30) for 30 days when it is compared with a control group. Low content of Hb was observed in the higher sub lethal concentration at 30 days of exposure.

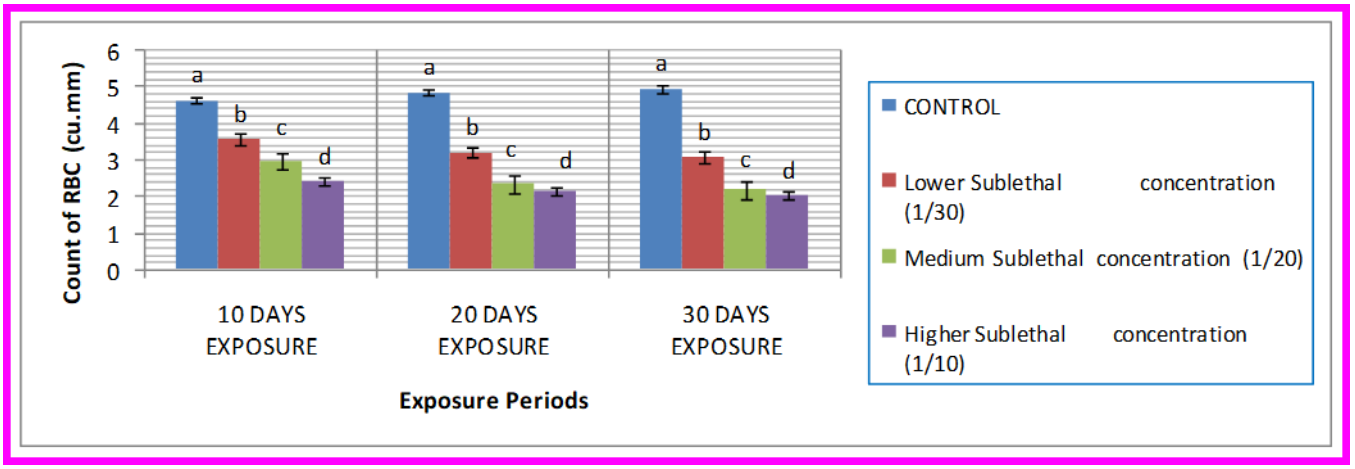

Fig.1.RBC count (cu. mm) of blood sample in freshwater fish *O. mossambicus* exposed to various sub lethal concentrations of Monocrotophos at various days of exposure.

Fig.1 shows decreased count of RBC in a blood sample of experimental fish *Oreochromis mossambicus* treated with various sub-lethal concentrations of cypermethrin (1/10, 1/20, and 1/30) for 30 days when it is compared with the control group. A highly decreased level of RBC count was observed in the higher sub lethal concentration at 30 days of exposure.

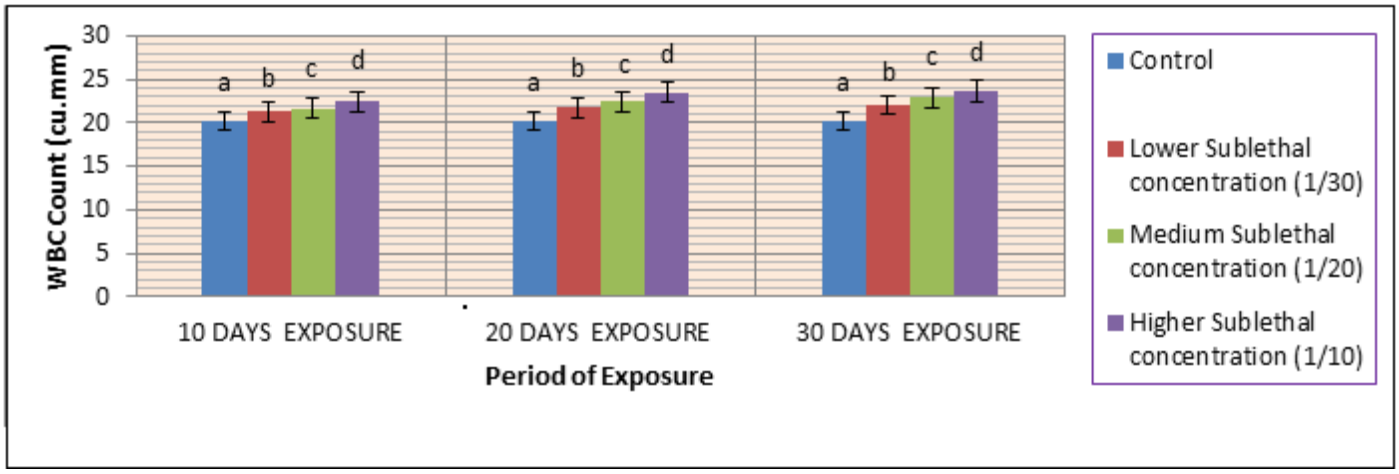

Fig.2.WBC count (cu. mm) of blood sample in freshwater fish *O. mossambicus* exposed to various sub lethal concentrations of Monocrotophos at various days of exposure.

Fig.2 shows an increased count of WBC in a blood sample of experimental fish *Oreochromis mossambicus* treated with various sub-lethal concentrations of cypermethrin (1/10, 1/20, and 1/30) for 30 days when it is compared with a control group. A highly increased level of WBC count was observed in the higher sub lethal concentration at 30 days of exposure.

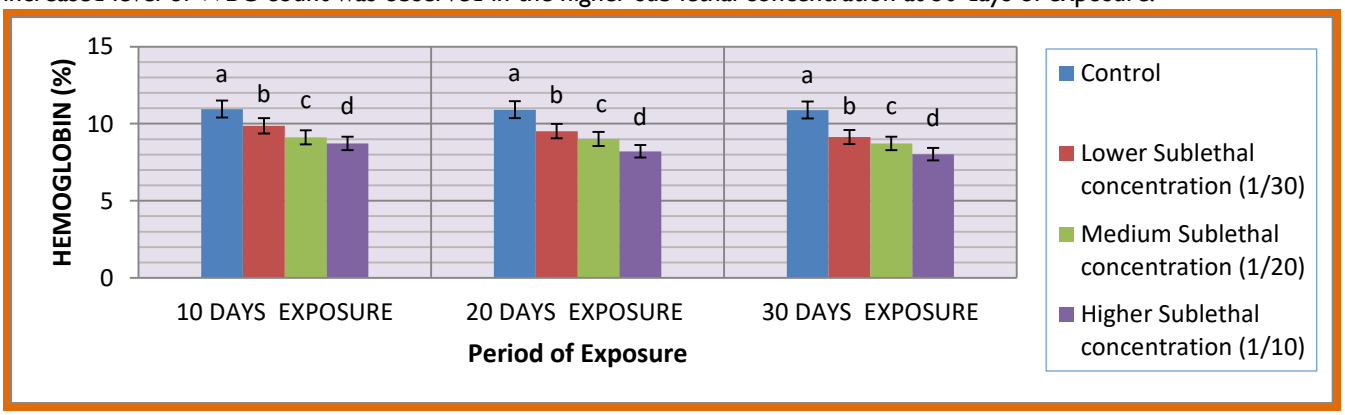

Fig.3.Hemoglobin (%) level of blood sample in freshwater fish *O. mossambicus* exposed to various sub lethal concentrations of Monocrotophos at various days of exposure.

Fig.3 shows decreased level of Hb in a blood sample of experimental fish *Oreochromis mossambicus* treated with various sub-lethal concentrations of cypermethrin (1/10, 1/20, and 1/30) for 30 days when it is compared with a control group. A highly decreased level of Hb was observed in the higher sub lethal concentration at 30 days of exposure.

5. DISCUSSION

Hematological tests have become one of the important diagnostic tools. Changes in the conditions of water quality and environment vary the blood composition of fish. In this study, a drastic reduction of RBC was observed in Monocrotophos treated fish *O. Mossambicus* at various sub lethal concentrations (1/10th, 1/20th, and 1/30th). Decreased level of RBC, noted in pesticide-treated fish, is due to the damage of Erythropoietic tissue[18]. Reddy and Bashmohideen[19] reported a reduced value of RBC after 48 hours of treatment to cypermethrin in fish *Cyprinuscarpio.* Similar results have been reported by Cazenave et al.[20] Reduction in RBC was reported for Cypermethrin treated *Labeorohita* and African catfish (*C.gariepinus*) treated with diazinon by Adedejiet al.[21] Reduction of RBC indicates hemorrhage and hemolysis in fishes on exposure to insecticide. Pesticide monocrotophos extend the toxic effect on fishes, the reduction in RBC count is observed in treated fishes, and similar reports were observed by Panigrahi et al.[22] and Agarwal et al.[23]. In earlier studies after exposure to sub-lethal doses of lead, the freshwater fish showed anaemia[24]. The decreased level of Hb observed in Monocrotophos treated fish, *O. Mossambicus* at various sub-lethal (1/10th, 1/20th, and 1/30th) conc. for the different duration (10,20 and 30 days) in the present study, may be due to increased destruction and lesser synthesis of hemoglobin due to the toxic effect of Monocrotophos. A significant reduction in Hb and RBC levels was observed by Adhikari et al., [25]. Reduction in hemoglobin content of treated fish may be an indication of a decline in Hb synthesis and also a reduction in oxygen-carrying capacity. Significant haemoglobin reduction and reduced RBC count in the blood observed in freshwater fish *O.mossambicus* exposed to pesticides were studied by Ramaswamy et al., [26] were, WBC level increased in Monocrotophos treated fish, *O. Mossambicus* for 30 days duration. Leucocytes value increased due to stimulation of the hemopoietic tissues. Declined level of hemoglobin and RBC count observed by an organophosphorus insecticide in Japanese quail was reported by Germanys-Katha waka et al.[27] A reduction in haemoglobin level was also observed in the Coho salmon exposed to pulp mill effluent[28]. The reduction of hemoglobin in the present experiment was supported by the toxic studyon Cyprinus carpio[29]. In the present study, the total WBC count of the Monocrotophos treated fish, *O. Mossambicus*, has remarkably increased at various sub lethal concentrations (1/10,1/20 and 1/30) and different exposure periods. The same results were observed by Venkataraman et al.[30] Increasing WBC count has been considered to be an adaptation method of animals to meet stressful conditions. A significant decrease was observed in the WBC count of *Channapunctatus* after exposure to various sub lethal concentrations of ammonia[31]. Dabrowaska& Wlasow[32] have also observed a decrease in WBC counts in *Cyprinuscarpio*. Hickely[33] observed that Leucopenia has a specific response of toxic stress related to the activities on fishes. The present experiment indicates that the pesticide stress stimulates the white blood cells to produce more at the time of exposure period. The present study concludes that Hematological changes are observed in the blood RBC, WBC, Hemoglobin of freshwater fish *Oreochromis mossambicus* by Monocrotophos depending upon the pesticide toxicity, dose-response, an exposure period of the fish.

6. CONCLUSION

Fish *Oreochromis mossambicus* treated to various sub-lethal concentrations of monocrotophos showed a decreased level of RBC, WBC, and increased level of hemoglobin at the end of the exposure period compared to a control group. The present experiment concluded that above the safe level uses of monocrotophos disturbs Hematological parameters like RBC, WBC, and Hemoglobin, and it may reduce the survival ability of the fish *Oreochromis mossambicus.* The experiment results provide awareness on safe level usage of Monocrotophos and it is recommended that the unsafe uses of monocrotophos should be restricted.

7. ACKNOWLEDGEMENT

The authors of the present work wish to thank the authorities and supporters of Government Arts College for Men, Krishnagiri, Tamilnadu for their help.

8. AUTHOR CONTRIBUTION STATEMENT

Mr.Solaiappan.A conceptualized and gathered the data about this work. Dr Prakash Sahaya Leon. J analyzed these data and necessary inputs were given towards the designing of the manuscript. All authors discussed the methodology, results and contributed to the final manuscript.

9. CONFLICT OF INTEREST

There is no conflict of interest.

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