

Application of Micronucleus Assay for Assessment of Profenofos Genotoxicity and its Mitigation through *Nyctanthes arbortristis* Leaf Extract in Mice (*Mus musculus*)

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ABSTRACT: The micronucleus test is always included in many genotoxic test guidelines for long time in many classes of chemicals, e.g., pharmaceutical chemicals, agricultural chemicals, food additives. The most commonly used agrochemical profenofos has adverse effect among human population. The dependence of people on the pesticides have may effect various organ system like respiratory system, reproductive system, etc. and also leading to genetic toxicity. In present work the mitigative effect of *Nyctanthes arbortristis* leaves were evaluated against genotoxic damage induced by Profenofos on PCEs and NCEs in mice bone marrow cells using Micronucleus test, the result shows that incidence of Mn formation in Profenofos treated group were significantly higher than the control .While concurrent treatment with *Nyctanthes arbortristis* leaves extract with Profenofos shows significantly lower than Profenofos treated group and almost equivalent upto the control level.

Keywords: Profenofos, *Nyctanthes arbortristis*, Micronucleus, Bone Marrow, Polychromatic erythrocytes, Normochromatic erythrocytes.

INTRODUCTION

India is an agricultural country where more than 80% population depends on the agriculture. With increase in human population, the problem to feed is becoming more and more difficult with every year. Increase in global food demand has resulted in a significant increase the use of agrochemicals in agriculture. Profenofos is a common organophosphate pesticides use for various agricultural and household purposes to control insects and other pests (IPCS, 1990; U.S. EPA, 1997). Agricultural mechanization has shifted from the use of natural pest control to that of synthetic pesticides due to rapidity and potency of their actions. Environmental hygiene and safety had benefited from pesticide use but also suffered adverse effects on non-target organisms including human due to uncontrolled use (Costa *et al.*, 2018). Many countries have focused on health effects of pesticides and prevention. As the methods of activity for pesticides are not species- specific, worries have been raised about environmental threat related with their exposure through different ways such as residues in diet and drinking water. Over utilization of pesticides may prompt the damage of biodiversity.

Micronucleus is a distinct and objective test of fragmentation of chromosome and any increase in their frequency over the control one (Gutierrez- Enriquez and Hall 2003), is a direct evidence of the clastogenic effect of the chemical administered to the test animal. It is a reliable, rapid and sensitive assay method for screening of potential clastogenes (Schmid, 1976; Das and Swain 1982; Das, 1984). Through the micronucleus test, investigate the chromosomal instability in humans who have mutations in genes which are needed for repair of DNA damage, as in the case of Fanconianemia (Zunino *et. al.*, 2001).

Various researches have shown that herbs, fruits and vegetables play an important role against toxicity of chemicals (Verma and Kumari 2022). It has been suggested that there is inverse relationship between the dietary intake of antioxidants rich foods and decrease in the incidence of genotoxicity. Therefore, the present work is designed to investigate the genotoxic effect of Profenofos in bone marrow cells of mice using Micronucleus test, so that incidence of micronuclei formation in polychromatic erythrocytes and normochromatic erythrocyte by the toxicity of profenofos can be assessed and also to mitigate its effect by *Nyctanthes arbortristis* leaves extract.

MATERIAL AND METHODS

4-6 week old Swiss albino mice *Mus musculus* with an average body weight of 25gm-30gm was used as test animal. The animals were divided into 4 groups and

treatment procedures are described in the Table 1, Each group contains 6 mice duration of the treatment was 30 days. Profenofos was used as toxicant and freshly collected, shed dried and powdered leaves extract of *Nyctanthes arbortristis* has used as mitigating agent.

Table 1: Summary of the treatment protocol.

Sr. No.	Experimental variants	Symbol	Dose
1.	Control	C	No dose
2.	Profenofos	P	1 mg/ml
3.	<i>Nyctanthes arbortristis</i> leaf extract	N	50 %
4.	Profenofos + leaf extract	P+N	As 1 and 2

After completion of treatment the mice were sacrificed by cervical dislocation, slides were prepared by method suggested by Schmid (1976) with slight modification (Das and Kar 1986; Salamone and Heddle 1983). Slides were screened, micronuclei present in polychromatic erythrocytes were stained blue and normochromatic erythrocytes were stained pink. Approx 1000-2000 PCEs & NCEs were counted per animal of each experimental group. Statical analysis was done using T-test.

RESULT

To evaluate the incidence of polychromatic erythrocytes and monochromatic erythrocyte, the micronucleus test was done for all 4 experimental variant.

Control group had a total of 11 (0.09 ± 0.02) micronucleus among which polychromatic erythrocytes were 7 ($0.11\% \pm 0.04$) and normochromatic erythrocyte were 4 ($0.06\% \pm 0.03\%$) This result showed very low frequency of Micronucleated erythrocytes in the control group.

In the another group of mice only profenofos was administered, this group had total Mn of $82(0.73 \pm 0.07)$ comprising PCE 63 (1.08 ± 0.13) of and NCE 19 (0.34 ± 0.07). This result showed highly significant value in comparison of control. Thus, profenofos causes damages to cells and leads to formation of Mn in highest frequency.

Nyctanthes arbortristis leaf extract was given to separated group of animal and noticed a total of $11(0.09 \pm 0.08)$ % micronucleus, Among these PCEs 7 ($0.11 \pm$

0.03) and NCEs were 4 (0.06 ± 0.03). These values were about equal to the control group and were insignificant. When *Nyctanthes arbortristis* leaf extract was given concurrently with profenofos a total of 25 (0.22 ± 0.03) Mn was obtained. Among which PCEs were 18 ($0.31\% \pm 0.07$) and NCEs were 7 (0.12 ± 0.04). The result of this group concluded that leaves extract of *Nyctanthes arbortristis* was much effective in decreasing the number of Mn induced by profenofos. The occurrence of micronucleus was comparatively higher in PCE in all four groups.

DISCUSSION

The results show that profenofos is potent enough to induce micronucleus in the cell. The present finding also show that concurrent administration of *Nyctanthes arbortristis* leaf extract could minimize the profenofos induced genotoxicity.

The micronucleus test in bone marrow and peripheral blood erythrocyte is one of the best established in vivo cytogenetic assay in the field of genetic toxicology providing the convenient and reliable index of both chromosomes breakage chromosomes loss (Fench, 2000; Nagy *et al.*, 2021). During micronucleus analysis, some authors have observed the occurrence of other nuclear abnormalities suggesting that they must be taken into consideration along conventional micronuclei analysis. Such abnormalities are related to cell division failure sand death process, so will as to genotoxicity and or mutagenicity (Da Silva Suza and Fontanetti 2006).

Table 2: Frequency of Micronucleated polychromatic erythrocytes (PCEs) and Normochromatic erythrocytes (NCEs) in the bone marrow cells of profenofos (P), *Nyctanthes arbortristis* treated (N) group of mice for 30 days duration.

Treatment	PCEs			NCE			PCEs + NCEs		
	Score	Mn	% \pm S.E	Score	Mn	% \pm S.E	Score	Mn	% \pm S.E
Control	6000	7	0.11 ± 0.04	5920	4	0.06 ± 0.03	11920	11	0.09 ± 0.02
P	5780	63	1.08 ± 0.13^a	5432	19	0.34 ± 0.07^a	11212	82	0.73 ± 0.07^a
N	6228	7	0.11 ± 0.03	5832	4	0.06 ± 0.03	12060	11	0.09 ± 0.08
P+N	5643	18	0.31 ± 0.07^{bc}	5412	7	0.12 ± 0.04	11055	25	0.22 ± 0.03^b

a, b and c indicate significant difference with corresponding value in the control, profenofos and *Nyctanthes arbortristis* respectively

The mitigative effect of *Nyctanthes arbortristis* leaf extract may be due to the Leaves contain mannitol, astringent, resinous substances, ascorbic acid, Leaves also contain the alkaloid nyctanthine along with nannitol, β -Amyrin β -Sitosterol, hentriacontane, benzoic acid, astragalol, nicotiflorin, oleanolic acid, nyctanthic acid, friedelin and lupeol (Kumari *et. al.* 2022) These flavonoids, tannins, glycosides as phenolic compounds and these phenolic compounds are antioxidant agent which shows free radical scavenging activity (Rani *et al.*, 2012).

CONCLUSION

From above discussion it is concluded that Profenofos is a potent genotoxic agent which induced micronuclei formation and *Nyctanthes arbortristis* leaves can mitigate its toxicity due to presence of antioxidant richness. Therefore, we must supplement our diet with antioxidants rich herbs. So that toxicity of various pesticides or chemicals can be minimized.

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