The Journal of Phytopharmacology 2021; 10(5):294-297
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2021; 10(5): 294-297
September- October
Received: 09-07-2021
Accepted: 17-08-2021

doi: 10.31254/phyto.2021.10504

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Evaluation of concurrent exposure of lower concentrations of lead and endosulfan on apoptosis by scatter pattern of flow cytometry

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ABSTRACT

The effect of repeated exposure of lower doses of lead and endosulfan were evaluated on apoptosis in male wistar rats. Rats of group I served as untreated control. Group II received drinking water with lead as lead acetate @100 ppm (Pb100). Group III was given feed containing technical grade endosulfan @ 10 ppm (E10). Group IV was exposed to Pb (100) +E (10). Splenocytes were analysed for estimating apoptotic percentage in rats. The results suggest that apoptotic percentage was not changed in the lower doses of endosulfan and lead when administered alone and also in combination in the present study.

Keywords: Lead, Endosulfan, Apoptosis.

INTRODUCTION

Lead constitutes one of the major environmental pollutants in developing countries [1]. Endosulfan comes under cyclodiene group of organochlorine pesticides used in agriculture. It is used for applications on vegetables and non-food crops such as cotton and tobacco. This colourless solid has emerged as a dangerous agrichemical due to its acute toxicity [2]. A dose of 10 ppm of endosulfan was tested to be non-toxic dose [3]. Since multiple-chemical exposure is supposed to represent an accurate picture of the human and animal chemical toxic burden, one chemical may change the effect of the other by changing its kinetics and/or dynamics in a co-exposure condition. In view of the amplified use of endosulfan for agriculture and increased levels of lead in ground water and environment, co-occurrence of lead and endosulfan seems to be accurate and coincidence contact of human and animals to these chemicals could be potentially unsafe.

Human and animals may be contacted with lead and endosulfan parallelly. The interaction resulting from the repeated exposure of lead and endosulfan cannot be assumed to be less hazardous. Hence the present study was aimed to estimate whether repeated co-exposure to lead at lower dose level through drinking water and to dietary endosulfan at lower dose could change the effect developed by each compound on apoptosis in male wistar rats.

MATERIALS AND METHODS

Colony-bred adult male albino Wistar rats (70-90g; 4-5 weeks age) were obtained from Laboratory Animal Resource Section, Indian Veterinary Research Institute, Izatnagar. As per the Institute Animal Ethical Committee guidelines they were kept under standard managemental environment. Four groups of six rats were used for the study. Rats of group I served as untreated control whereas Group II was given drinking water containing lead as lead acetate @100 ppm (Pb100). Group III was given feed containing technical grade endosulfan @ 10 ppm (E10). Group IV was given Pb (100) +E (10). All the treatments were administered daily for 28 days. Rats of all the groups were sacrificed after 28 days of treatment and splenocytes were isolated to find out apoptosis based on scattering pattern through flow cytometry.

Spleen was removed from rats using ice cold PBS and placed on a sterile, nylon mesh pre-wetted with PBS. Capsule of the organ was removed using forceps and needles. The organ was disintegrated into pieces. With the plunger of a 10 ml glass syringe, the tissue was sent through the mesh into a petridish containing PBS. Once the entire tissue passed out, the cell suspension was pipetted 4 -5 times to break large cell clumps. The suspension was collected in 15 ml centrifuge tube and the tube was kept to stand on ice. After 5 min, the top 12 ml of the suspension was collected into another tube and cells were pelleted by centrifuging (220 g for 10 min). Cell pellets were resuspended in PBS and layered over histopaque-1077 at a ratio of 1:1. The tubes were centrifuged at 1600 rotation per minute for 40 min and the interface ring rich in splenocytes was obtained. Washed cells were adjusted to give the anticipated cell concentration in RPMI-GM after assessing the cell count.

Splenocytes were cleaned with ice cold PBS and then fixed with 4% PFA for 30 min at room temperature. The cells were washed thrice with PBS and finally suspended in PBS. In cell suspension, propidium iodide (PI) was added to achieve a final concentration of 32 µM. Five min after staining, cells were checked at a cell throughput rate of 10,000 cells / gate using flow cytometer (Becton Dickinson, USA). Forward (FSC for size) and side (SSC for granularity) scatter counts were recorded using excitation wave length of 448 nm and emission wave length of 530 nm.

Apoptosis were calculated based on their scattering pattern. Cells showing less FSC and high SSC are recognised as apoptotic cells and recorded as percentage apoptotic cells

(n =10,000 events). Results have been communicated as mean ± SEM. The data were analyzed by ANOVA with Duncan's multiple comparisons [4].

RESULTS AND DISCUSSION

Cells in living organisms are now found to have the capacity to stimulate an enzyme cascade, terminating in the programmed death of the cell. An important aspect of apoptosis is that it happens without inflammation and facilitates the elimination of cells with minimal interruption to the surrounding cellular environment [5]. Apoptosis is the equal and counterbalance to mitosis in cell population. Apoptosis is also important for governing immune response [6]. Spleen is one of the principle sites for the origination of most primary immune responses, for B lymphocyte stimulation and the production of antibodies [7]. The mechanism of apoptosis stimulated by endosulfan was speculated as uncoupling of oxidative phophorylation-excess ROS production-GSH depletion-oxidative stress- release of cytochrome C and other apoptosis related proteins in the cytosol which induced apoptosis [8]. Endosulfan at 25, 50 or 250 µM was found to produce early apoptotic cell death in thymocytes *in vitro* [9]. Though there are no reports on induction of apoptosis by endosulfan in rats, induction of apoptosis in splenocytes of chickens has been reported [10]. In the present study, endosulfan at lower concentration (10 ppm) did not produce significant change as compared to control in apoptotic percentage (Fig.1) and the percentage of apoptosis cells were found to be 0.29% (Table.1).

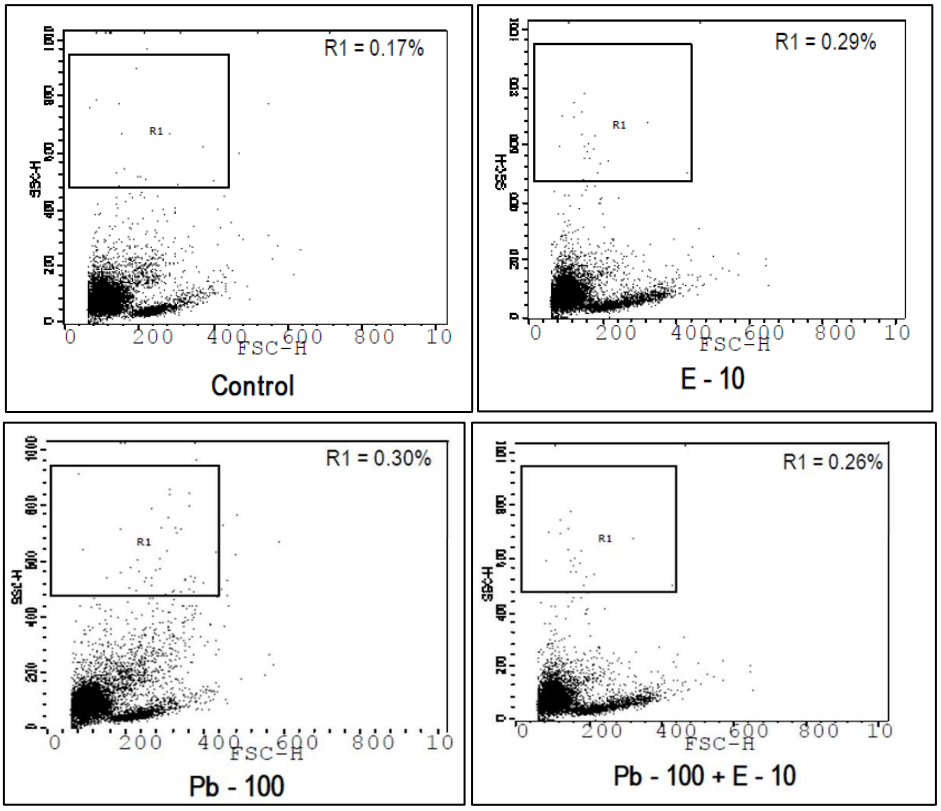

Figure 1: Representative histogram of flow cytometry output depicting percent apoptotic cells (R1) in splenocytes on staining with propidium iodide (FSC-Forward scatter channel; SSC-Side scatter channel)

Table 1: Evaluation of 28 days treatment with lead, endosulfan and their combination on percentage apoptosis in splenocytes of rats (Mean ± S.E.M; n=6; P≤0.05)

Groups	Apoptosis (%)
Control	0.17±0.03
Pb-100	0.30±0.04
E-10	0.29±0.02
Pb-100+E-10	0.26±0.07

Values are Mean ± SE of six rats. Pb-100 denotes lead 100 ppm and E-10 indicates endosulfan10 ppm. Lead and endosulfan were given to rats in water and feed, respectively.

The apoptogenic potential of lead has been demonstrated in various studies [11] [12][13]. Chronic exposure to lead acetate produced cytotoxic effect in male rats, which seemed to be related to an apoptotic process [14]. Lead acetate administered intraperitoneally at 15 mg/kg daily for 7 days was shown to produce neurotoxicity which may partly be due to apoptosis [15]. In the present study, lead at lower concentration (100 ppm) did not produce significant change as compared to control in apoptotic percentage and the percentage of apoptosis cells was found to be 0.30% (Table.1).

Detectability of the non-toxic effects of the single compounds can be changed by the interactions with one or more other ones by oral route, which can lead to unexpected health consequences. Hence detectability of non-toxic dose effects of endosulfan and lead when given alone may be changed by the combined exposure by oral route in apoptotic percentage in male rats. This in turn may lead to serious health hazards as these compounds are ubiquitous in nature. Antagonistic activity was suggested in group treated with endosulfan and carbaryl in higher dose combination as compared to high dose internal control in WBC and in lymphocyte counts [16]. Another study suggested that lower concentrations of lead and endosulfan when given in combination did not change the levels of general biochemical parameters in wistar rats [17]. Another study reported that lead and cadmium in toxic combination showed intermediate results on UDP-glucoronyl transferase enzymes when compared to the compounds when given alone [18]. A study also suggested that lower concentrations of lead and endosulfan when given in combination did not change the levels of drug metabolizing enzymes in wistar rats [19]. In the present study, there were no major interaction in terms of synergism and antagonism in the combination of lead and endosulfan at nontoxic doses (Table.1). Results also show that nontoxic concentrations of lead and endosulfan when given in combination did not change the oxidative stress indices in rats [20].

CONCLUSION

In conclusion, the lower concentrations of endosulfan and lead when given alone and in combination did not vary in apoptotic percentage analyzed in the study through forward and side scatter pattern.

Acknowledgements

The authors express their greatfulness to the Director, Indian Veterinary Research Institute, Izatnagar for providing essential facilities for conducting this study.

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HOW TO CITE THIS ARTICLE
Ranganathan V, Malik JK, Rao GS. Evaluation of concurrent exposure of lower concentrations of lead and endosulfan on apoptosis by scatter pattern of flow cytometry. J Phytopharmacol 2021; 10(5):294-297. doi: 10.31254/phyto.2021.10504