

## *In-vivo* Evaluation of Clastogenic and Aneugenic potential of Palmitoyl-mono-Ethanolamide (PEA)

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Received: 17 Mar 2024; Accepted: 20 Apr 2024; Published: 28 Apr 2024

**Citation:** Narendra S Deshmukh, Kanhere Jaideep, Sedatrul Muntha, et al. *In-vivo* Evaluation of Clastogenic and Aneugenic Potential of Palmitoyl-mono-Ethanolamide (PEA). J Med - Clin Res & Rev. 2024; 8(4): 1-4.

### Keywords

Palmitoyl-mono-Ethanolamide, Clastogenicity, Aneugenicity.

### Introduction

The pharmacological properties of the autacoid PEA are well documented, with over 350 papers describing its anti-inflammatory, anti-viral, analgesic, antiepileptic and neuroprotective effects [1]. PEA's actions at CB1, GPR55 [2,3] and other receptors in the central nervous system have been shown to modulate excitatory glutamatergic signalling and dopaminergic activity via the mesolimbic pathway, with therapeutic relevance for cognition, contextual memory and emotional regulation [3].

This research has led to a proliferation of non-licensed PEA supplements, which raises safety concerns because the available safety data is rather limited. The most relevant research in this area, including rodent [4,5] and dog studies [6,7] is undoubtedly helpful, but important gaps in PEA's safety profile remain.

The Nestmann paper [4] includes the Ames test, an *in vitro* mammalian cell micronucleus test and acute, 14- and 90-day oral toxicity studies in rats. There was no evidence of cytotoxicity or genotoxicity. The acute oral LD<sub>50</sub> was >2,000 mg/kg body weight, and the 14-day and 90-day sub-chronic oral toxicity studies revealed no treatment-related adverse events. The no-observed-adverse-effect level (NOAEL) of micronized PEA was 1,000 mg/kg body weight /d, the highest dose tested. Deshmukh et al. [5] demonstrated a NOAEL for maternal toxicity, embryotoxicity, fetotoxicity, and teratogenicity >1,000 mg/kg body weight/d, equivalent to a human dose of greater than 9.7 g/d.

No significant or dose-related adverse effects of PEA have been reported in animal models [4-7] or in human studies [8,9] to date.

However, and especially concerning given the rapid commercial development of PEA, there is not yet sufficient evidence regarding possible genotoxicity.

The genotoxicity testing strategy in the EFSA Scientific Committee opinion [10] is designed to investigate the genotoxic potential of substances through the detection of gene mutations, structural chromosomal aberrations and numerical chromosomal aberrations. The testing strategy is a stepwise approach, beginning with a basic battery of *in vitro* tests, comprising of a bacterial reverse mutation assay [Ames Test, OECD TG 471, end-point: gene mutations]; and an *in vitro* mammalian cell micronucleus (MN) test (OECD TG 487, endpoints: clastogenicity and aneugenicity) [11].

Apart from the two *in vitro* mutagenicity tests described by Nestmann [4], the possible clastogenic and aneugenic properties of PEA have not been adequately studied; and no *in vivo* micronucleus test has been reported in the literature. An *in vivo* study was therefore initiated to confirm and substantiate the *in vitro* PEA studies.

Our study evaluated the genetic toxicology of PEA in terms of clastogenicity and aneugenicity in an erythrocyte micronucleus test in mice dosed orally at of 2000 mg/kg body weight. The study was conducted in compliance with the Organization for Economic Cooperation and Development (OECD) test guideline number 474 and the OECD principles of GLP; and carried out at the OECD GLP certified test facility of Intox Pvt. Ltd., Pune, India.

### Methods

The *In vivo* Micronucleus Test for Palmitic Acid Mono Ethanolamide (CAS no. 544-31-0) was performed in mouse peripheral blood cells. A white crystalline powder of PEA,

manufactured by Shilpa Medicare Ltd., Raichur, Karnataka, India in April 2020 (Batch Number PML220002G) with the active ingredient to be >99% w/w was supplied by Gencor Pacific Limited, Hong Kong, along with its certificate of analysis. The PEA was uniformly suspended in analytical grade water by using Tween 80 as a wetting agent at 0.2% w/v. The dosing formulations were prepared immediately prior to administration, using the same method that was validated for ensuring homogeneity in previous GLP studies performed at Intox, which had demonstrated that PEA was stable in such formulation for 48 h at 22 ± 3 °C.

### Animal Welfare

The study was performed as per the Study Plan approved by the Institutional Animal Ethics Committee. The meeting was held on 12 January, 2022 and Form B12/21007 for the present study was approved. The relevant certificate of approval has been maintained at the Test Facility.

### Selection of Test Doses

A preliminary study was performed in the same laboratory (Intox Pvt Ltd), using the same species, strain, sex, and treatment regimen to be used in the main study, as required by the OED TG 474. Six Swiss albino mice, three male and three female, bred and reared at Intox Pvt Ltd, were dosed with PEA by oral gavage for two consecutive days at a dose of 2000 mg/kg body weight. The only adverse effect of treatment was transient hypoactivity in mice of both sexes following the second dose of PEA.

Since there was no previous evidence of observable significant toxicity at 2000 mg/kg body weight, identified as a ‘limit dose’ by the OECD TG 474, and there was no sex-difference observed in the manifestation of the signs of hypoactivity, it was decided to conduct the main study as a ‘Limit Test’ at a single dose level at the ‘limit dose’ and that only in male mice.

### Main Study

Five male Swiss albino mice, bred and reared at Intox Pvt Ltd, were administered with PEA, formulated in analytical grade water with 0.2% Tween 80, by oral gavage at the dose of 2000 mg/kg body weight. A concurrent vehicle control group of five male mice was administered with 10 mL/kg of analytical grade water

with 0.2% Tween 80, while another concurrent positive control group of five male mice was administered with cyclophosphamide monohydrate at the dose of 15 mg/kg body weight. The mice were treated for two consecutive days, at an interval of about 24 hours.

All mice were observed for signs of toxicity following the treatment and were sacrificed at 45 hours after the last treatment. The animals were monitored for mortality, clinical signs and body weight. Blood from each mouse was collected, fixed and subsequently stained with anti-mouse CD71 and anti-rodent CD61 antibodies. About 21,000 polychromatic erythrocytes (PCEs, i.e. immature erythrocytes) per animal were examined using a flow cytometer to detect the incidence of micronucleated PCEs (MN-PCEs). In addition, a proportion of immature erythrocytes was assessed for each animal as a measure of potential toxicity.

## Results

### Survival, Clinical Signs, Body Weights

All mice survived treatment with PEA. Signs of transient hypoactivity were observed in three mice treated with PEA following its second dose at 2000 mg/kg body weight. PEA did not adversely affect the body weight of any of the treated mice.

### Incidence of PCEs (%)

The incidence of PCEs (CD-71 positive immature erythrocytes) in mice treated with PEA was 1.34 ± 0.19%. This was comparable (p>0.05) with the PCE incidence of 1.49 ± 0.16 % in the vehicle control group. In contrast, in mice treated with positive control item Cyclophosphamide monohydrate, there was a statistically significant (p<0.05) reduction in the incidence of PCEs, with the mean found to be 0.76 ± 0.07%. This was 49% lower than that of the vehicle control group (Table 1).

### Incidence of MN-PCEs (%)

The incidence of micronucleated PCEs (MN-PCEs, mean ± SD) in mice treated with two doses of PEA at 2000 mg/kg was 0.16 ± 0.01%, while that in the vehicle control group was 0.14 ± 0.02%. The means were statistically comparable (p>0.05).

The incidence of MN-PCEs in mice treated with the positive control item Cyclophosphamide monohydrate was 0.87 ± 0.07%,

**Table 1:** Proportion of Immature Erythrocytes and Micronucleated Erythrocytes.

Group	Dose (per kg bwt)	Description	Incidence	
			% PCE	% MN-PCE
Vehicle Control (Analytical grade water with 0.2% Tween 80)	10 mL	Mean	1.49	0.14
		± SD	0.16	0.02
		n	5	5
Treatment (PEA – Palmitic Acid Mono Ethanolamide)	2000 mg	Mean	1.34	0.16
		± SD	0.19	0.01
		n	5	5
Positive Control (Cyclophosphamide monohydrate)	15 mg	Mean	0.76	0.87
		± SD	0.07	0.07
		n	5	5

PCE – Polychromatic (immature) erythrocytes; MN-PCE – Micronucleated PCEs; Sampling time: 45 h after last dose

\*mean differs from the vehicle control mean statistically at p<0.05.

which was significantly lower ( $p < 0.05$ ) than the control mean (Table 1).

## Discussion

Two daily consecutive treatments with PEA in mice at the limit dose of 2000 mg/kg body weight did not cause death or any overt signs of toxicity. The transient hypoactivity observed following the second dose was consistent with findings observed in the preliminary studies. The oral LD50 of PEA in mice was previously reported to be  $> 4$  g/kg body weight (18) and the findings of the present study were consistent with this.

The incidence of PCEs in mice treated with PEA was comparable ( $p > 0.05$ ) with the PCE incidence in the vehicle control group, indicating an absence of haemopoietic toxicity. The positive Cyclophosphamide monohydrate controls were as expected.

The incidence of micronucleated immature erythrocytes (% MN-PCE) in the peripheral blood of treated mice matched the vehicle control group ( $p > 0.05$ ), indicating a null effect. Again, the positive Cyclophosphamide monohydrate controls were as expected, confirming the sensitivity of the test system and validity of the assay.

As PEA is increasingly being used in the supplement space, which is neither supervised nor adequately monitored, and by consumer groups which include children and pregnant women, the issue of potential genotoxicity was considered as a matter of urgency. This is not an abstract concern; natural supplements including herbs have been previously identified as having clastogenic and aneugenic properties [ie 12,13], and regulatory authorities have been compelled to respond appropriately.

The mammalian erythrocyte MN Test (OECD TG 474), covering the endpoints of structural and numerical chromosomal aberrations of erythroblasts, is the only validated assay for the *in vivo* follow-up of *in vitro* aneugenic compounds. The experimental protocol was standardised to evaluate MN formation in young erythrocytes (polychromatic) sampled in bone marrow and/or reticulocytes in peripheral blood cells of rodents. Newly formed micronucleated erythrocytes are identified and quantitated by staining followed by visual microscopic scoring. With positive results, kinetochore staining or FISH with a pan-centromeric probe is not mandatory to confirm aneuploidy *in vivo*. Automated analysis of micronuclei on cell suspensions using flow cytometry allows scoring of a large number of cells, reducing scorer subjectivity and increasing the statistical power.

Unmicronised, micronised, and ultra-micronised PEA formulations are absorbed following oral administration, with measurements of PEA in blood plasma in rats [14] and in humans [9,15]. Exposure of bone marrow to PEA has therefore been established. While the earlier *in vitro* mutagenicity studies provide some reassurance of absence of genotoxicity, our *in vivo* findings extend and confirm PEA's safety profile.

## Conclusion

Under the given conditions of this mammalian (*in vivo*) erythrocyte micronucleus test in mouse peripheral blood, the test item Palmitoyl Mono-Ethanolamide (PEA) is non-clastogenic and non-aneugenic.

## References

1. Keppel Hesselink JM, de Boer T, Witkamp R. Palmitoylethanolamide A Natural Body-Owned Anti-Inflammatory Agent Effective and Safe against Influenza and Common Cold. *Int J Inflamm*. 2013; 1-8.
2. Petrosino S, Schiano Moriello A, Cerrato S, et al. The anti-inflammatory mediator palmitoylethanolamide enhances the levels of 2-arachidonoyl-glycerol and potentiates its actions at TRPV1 cation channels. *Br J Pharmacol*. 2015; 173: 1154-1162.
3. Kramar C, Loureiro M, Renard J, et al. Palmitoylethanolamide Modulates GPR55 Receptor Signaling in the Ventral Hippocampus to Regulate Mesolimbic Dopamine Activity Social Interaction and Memory Processing. *Cannabis Cannabinoid Res*. 2017; 2: 8-20.
4. Nestmann, Earle R. Safety of micronized palmitoylethanolamide microPEA lack of toxicity and genotoxic potential. *Food Sci Nutr*. 2016; 5: 292-309.
5. Deshmukh, Narendra S, Silma Subah, et al. Palmitoylethanolamide prenatal developmental toxicity study in rats. *Int J Toxicol*. 2011; 40: 161-170.
6. Chiara Noli, Federica Della Valle M, Alda Miolo, et al. Efficacy of ultra-micronized palmitoylethanolamide in canine atopic dermatitis an open-label multi-centre study. *Vet Dermatol*. 2015; 26: 432-440.
7. Cordaro M, Siracusa R, Impellizzeri D, et al. Safety and efficacy of a new micronized formulation of the ALIamide palmitoylglucosamine in preclinical models of inflammation and osteoarthritis pain. *Arthritis Res Ther*. 2019; 21: 254.
8. Elizabeth Steels, Ruchitha Venkatesh, Eleanor Steels, et al. A double-blind randomized placebo-controlled study assessing safety tolerability and efficacy of palmitoylethanolamide for symptoms of knee osteoarthritis. *Inflammopharmacology*. 2019; 27: 475-485.
9. Gabrielsson L, Mattsson S, Fowler C. Palmitoylethanolamide for the treatment of pain pharmacokinetics safety and efficacy. *British Journal of Clinical Pharmacology*. 2016; 82: 932-942.
10. EFSA Scientific Committee. Scientific Opinion on Genotoxicity Testing Strategies Applicable to Food and Feed Safety Assessment. *EFSA Journal*. 2011; 9: 69. <https://doi.org/10.2903/j.efsa.2011.2379>
11. EFSA Scientific Committee Scientific Opinion on the guidance on aneugenicity assessment. *EFSA Journal*. 2021; 19: 27. <https://doi.org/10.2903/j.efsa.2021.6770>
12. Bernacki DT, Bryce SM, Bemis JC, et al. Evidence for an Aneugenic Mechanism of Action for Micronucleus Induction by Black Cohosh Extract. *Environ Mol Mutagen*. 2019; 60: 845-856.

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13. Pereira-Martins SR, Takahashi CS, Tavares DC, et al. In vitro and in vivo study of the clastogenicity of the flavone cirsiitakooside extracted from *Scoparia dulcis* L. Scrophulariaceae. *Teratog Carcinog Mutagen*. 1998; 18: 293-302.
  14. Stefania Petrosino, Marika Cordaro, Roberta Verde, et al. Oral Ultramicronized Palmitoylethanolamide Plasma and Tissue Levels and Spinal Anti-hyperalgesic Effect. *Front Pharmacol*. 2018; 25: 35-42.
  15. Briskey D, Mallard AR, Rao A. Increased Absorption of Palmitoylethanolamide Using a Novel Dispersion Technology System LipiSpense®. *J Nutraceuticals Food Sci*. 2020; 5: 3.