



Topical Polyethylene Exposure Induces Conjunctival Inflammation and Early Meibomian Gland Alterations in Wistar Rats

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Keywords

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Abstract

Nanoplastics are plastic particles smaller than 1000 nm formed from plastic degradation, including polyethylene (PE). These particles can enter the eye through environmental exposure or topical products and may induce oxidative stress, inflammation, and Meibomian gland dysfunction. Evidence regarding ocular toxicity from topical nanoplastic exposure remains limited. To evaluate the effects of topical polyethylene nanoplastic exposure on ocular surface inflammation and Meibomian gland histology, this experimental post-test only study used male *Rattus norvegicus* Wistar rats receiving topical polyethylene nanoplastic eye drops. Histopathological analysis with Hematoxylin–Eosin staining assessed PMN infiltration, normal Meibomian acini, acinar width, and gland obstruction. PMN infiltration in the palpebral conjunctiva increased significantly ($p=0.014$), while normal Meibomian acini decreased significantly ($p=0.000$). Other parameters showed no significant differences. Topical polyethylene nanoplastic exposure induces conjunctival inflammation and early Meibomian gland alterations.

INTRODUCTION

Plastic production continues to rise globally, leading to extensive environmental accumulation and degradation into micro and nanoplastic particles (Bajt, 2021; Chaisrihwun et al., 2023; Cunningham et al., 2023; Jaeger et al., 2024; Son et al., 2024). Polyethylene (PE) is one of the most frequently used plastic polymers due to its durability and broad industrial application (Hüffer et al., 2019; Schröter & Ventura, 2022; Sun et al., 2021). Fragmentation of PE generates nanoplastic (<1000 nm), which can disperse widely in air and water, facilitating unintentional exposure to human and animals (Eberhard et al., 2024; Zhu et al., 2023). Their nanoscale size and high surface reactivity allow cellular penetration, oxidative stress induction, and inflammatory responses, raising concern regarding their potential biological toxicity (Das, 2023; Wang et al., 2025; Wu et al., 2023).

The ocular surface is particularly vulnerable to environmental pollutants because of its direct exposure and limited protective barriers (Wang et al., 2025; Wu et al., 2023; Zhao et al., 2023; Zhou et al., 2022). Nanoplastic particles may interact with the tear film and ocular adnexa, including the Meibomian glands, which play a key role in maintaining tear film stability (Chang & Purt, 2021; Kopacz et al., 2021). Dysfunction of the Meibomian glands can result in lipid layer disruption, increased tear evaporation, and ocular surface inflammation (Chhadva et al., 2017; Dietrich et al., 2021; Mizoguchi et al., 2017; Obata, 2002; Tu et al., 2024). Previous studies have demonstrated inflammatory responses associated with microplastics; however, evidence specific to topical nanoplastic exposure and its effect on ocular tissues remains scarce (Tu et al., 2024; Wang et al., 2025; Wu et al., 2023).

Given the rising environmental prevalence of nanoplastics and the limited

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data regarding ocular toxicity, experimental studies are necessary to clarify potential pathological changes. Therefore, this study aims to evaluate the effect of topical polyethylene nanoplastic exposure on polymorphonuclear (PMN) cell infiltration in the cornea and palpebral conjunctiva, as well as structural changes of the Meibomian glands in Wistar rats. Findings are expected to contribute new insights into nanoplastic-induced ocular inflammation and support further risk assessment for environmental exposure.

RESEARCH METHODS

Ethical Approval and Experimental Design

This research was an experimental laboratory study using a *post-test only control group design*. The protocol received ethical approval from the Ethics Committee of the Faculty of Medicine, Universitas Katolik Widya Mandala Surabaya. Twenty-eight healthy male *Rattus norvegicus* Wistar rats, aged 6–8 weeks and weighing 150–200 g, were acclimatized for eight days and housed individually in controlled laboratory conditions (19.7°C; 44% relative humidity; 12-h light/dark cycle). Standard laboratory feed (Fur 594, Indonesia) 30 g/day and water were provided *ad libitum* in a 250mL bottle. The animals were randomly allocated into four groups (n = 7/group), consisting of one control group

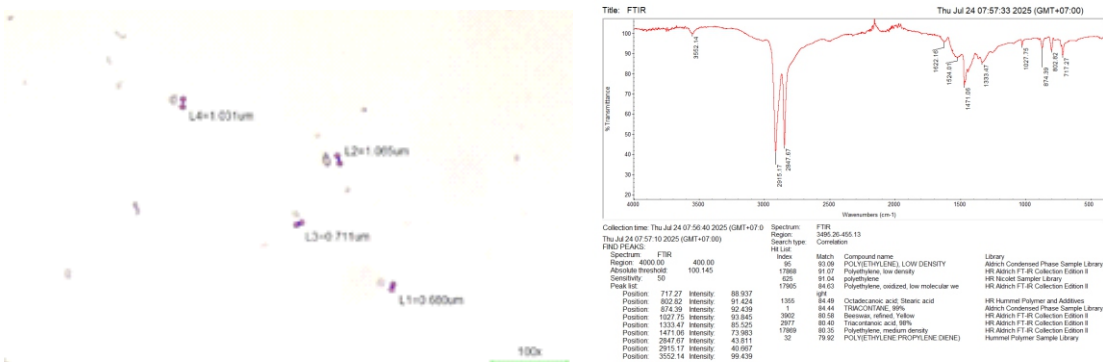
receiving sterile distilled water eye drops and three treatment groups receiving polyethylene nanoplastic suspensions eye drops.

Preparation and Administration of Polyethylene Nanoplastic

Low-density polyethylene (LDPE) nanoplastic powder measured $\leq 1 \mu\text{m}$ (CHEMAZONE, Toronto, Canada) was weighed using an analytical balance and dissolved in sterile distilled water to prepare concentrations of 0.5 mg/0.1 mL, 1 mg/0.1 mL, and 2 mg/0.1 mL. Each rat in the treatment groups received two drops ($\sim 0.1 \text{ mL}$) per day of the suspension topically into the conjunctival sac using a glass pipette, while the control group received the same volume of distilled water. Administration was performed once daily for 28 consecutive days.

Euthanasia, Tissue Collection, and Histopathological Processing – Evaluation

At the end of treatment, animals were euthanized using intraperitoneal injection of ketamine (300 mg/kg) and xylazine (30 mg/kg). The eyeballs and palpebrae were enucleated, rinsed with sterile saline, and fixed in 10% neutral buffered formalin. Tissues were processed using standard paraffin embedding, sectioned at 4–5 μm , and stained with Hematoxylin–Eosin (HE). Histopathological evaluation was performed using a light microscope at 400 \times magnification, and images were



Source: Primary Data Processed, (2025)

Figure 1
Microscopic Image of Nanoplastic Particles at 10x10 magnification (left) and Fourier Transform Infrared (FTIR) Test Results for Low Density Polyethylene (LDPE) Nanoplastic Particles (right)

captured automatically using Image Raster software. Observations for each variable were quantified in five random fields of view per specimen.

Outcome Measures and Statistical Analysis

The assessed outcomes included polymorphonuclear (PMN) cell infiltration in the cornea and palpebral conjunctiva, number of normal Meibomian gland acini, acinar diameter (µm), and degree of Meibomian gland obstruction. Data were processed and analyzed using IBM SPSS Statistics v25 (IBM Corp., Armonk, NY, USA). Normality was tested using the Shapiro-Wilk test, while homogeneity was evaluated with Levene's test. Parametric data were analyzed using One-Way Anova followed by LSD post-hoc test, whereas nonparametric data were analyzed using the Kruskal-Wallis test

followed by Mann-Whitney U where appropriate. A significance threshold of $p < 0.05$ was applied.

RESULTS AND DISCUSSION

Experimental Animals

A total of 28 rats were included at the beginning of the study; however, three animals died during the exposure period, resulting in 25 rats completing the experiment. Topical exposure to polyethylene nanoplastic suspensions produced histopathological changes mainly in the palpebral conjunctiva and Meibomian glands.

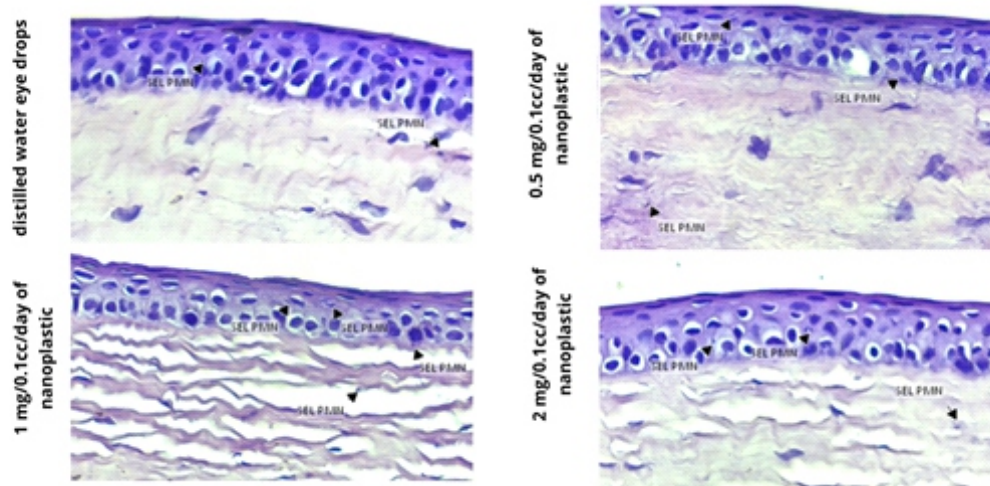
Characterization of LDPE Nanoplastic

The nanoplastic material used in this study consisted of low-density polyethylene (LDPE) particles with a size $\leq 1 \mu\text{m}$ (figure 1, left). Microscopic observation confirmed heterogeneous particle morphology

Table 1
Mean ± SD of ocular histological parameters following nanoplastic exposure

Parameter	Control group (X0)	Experimental group		
		X1	X2	X3
(Mean ± SD)				
Polymorphonuclear (PMN) cell count in the cornea	56.83±6.113	62.00±5.138	65.17±11.427	56.29±8.597
Polymorphonuclear (PMN) cell count in the palpebral conjunctiva	65.83±9.559	74.83±11.805	88.83±14.634	83.29±10.388
Normal meibomian gland acini count	68.00±4.243	46.33±1.506	67.33±3.830	50.29±7.910
Meibomian acinar cell diameter	7.3617±0.59925	7.1017±0.75184	8.2883±3.20505	6.7657±0.71914
Meibomian duct diameter	180.0967±96.31801	146.1983±63.14173	93.6000±35.08228	125.8214±35.06799

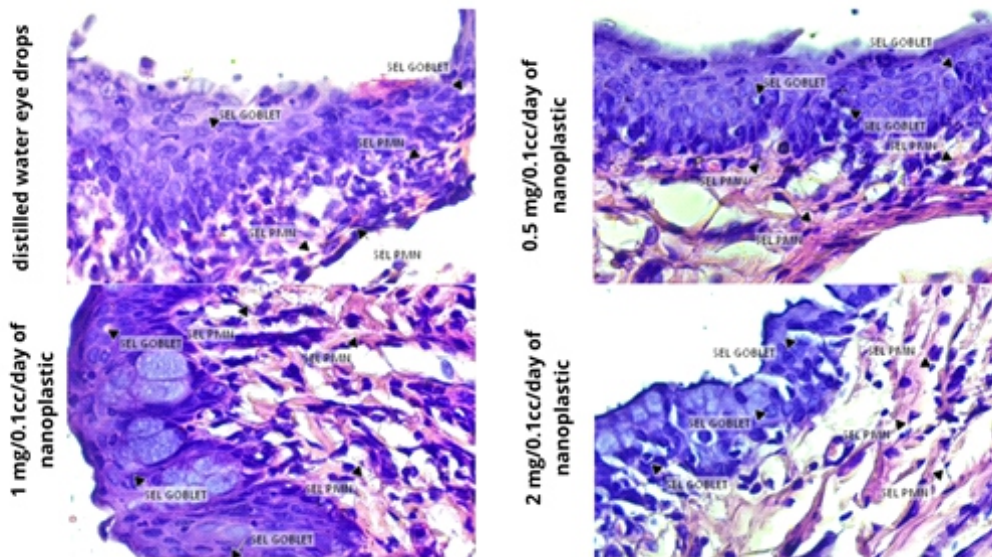
Source: Primary Data Processed, 2025



Source: Primary Data Processed, (2025)

Figure 2

Microscopic appearance of Polymorphonuclear (PMN) cells in the cornea stained with Hematoxylin-Eosin (H&E) using a Nikon Eclipse Ei microscope at 400x magnification.



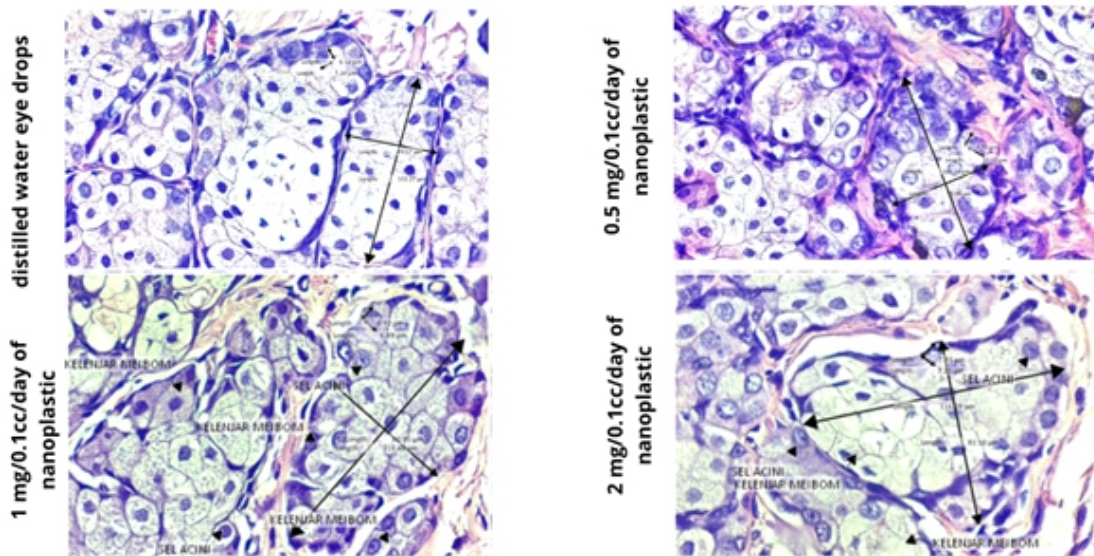
Source: Primary Data Processed, (2025)

Figure 3

Microscopic appearance of Polymorphonuclear (PMN) cells in the palpebral conjunctiva stained with Hematoxylin-Eosin (H&E) using a Nikon Eclipse Ei microscope at 400x magnification.

within the nanoplastic size range, while Fourier Transform Infrared (FTIR) analysis demonstrated characteristic polyethylene absorption bands consistent with LDPE, confirming the chemical identity of the preparation (figure 1, right). Although polyethylene is generally considered chemically inert, nanoplastic-sized particles exhibit increased surface area and enhanced interaction with biological tissues. Following topical application, LDPE nanoplastics

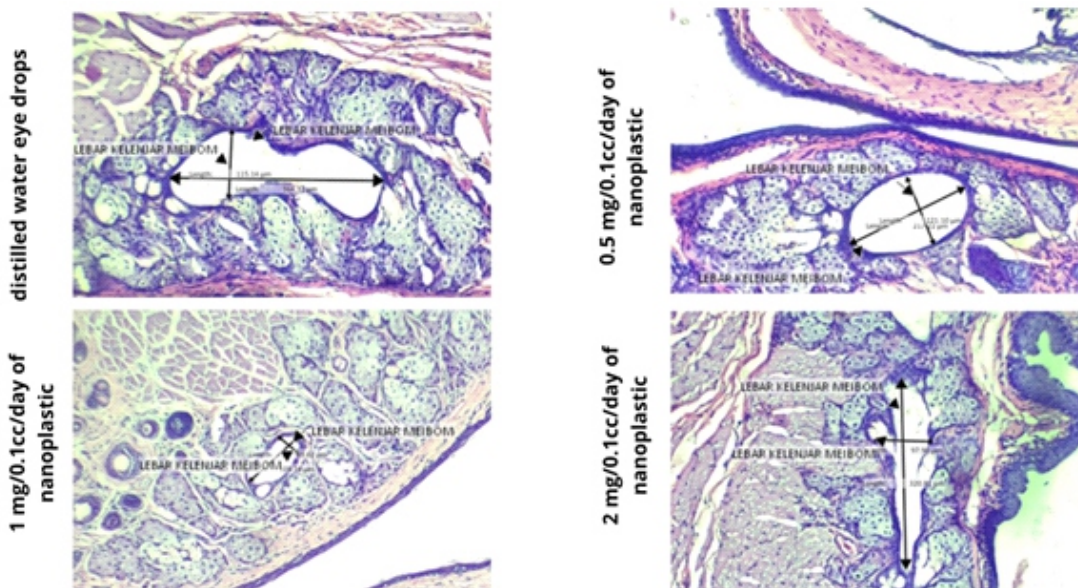
may persist on the ocular surface and conjunctival mucosa, potentially inducing oxidative stress and inflammatory responses. This mechanism may explain the increased polymorphonuclear cell infiltration in the palpebral conjunctiva and the reduction in normal Meibomian gland acini observed in this study, despite the absence of significant changes in acinar or ductal diameters, suggesting early or subclinical glandular involvement.



Source: Primary Data Processed, (2025)

Figure 4

Microscopic appearance of Meibomian gland duct acini and Meibomian acinar cell diameter stained with Hematoxylin-Eosin (H&E) using a Nikon Eclipse Ei microscope at 400x magnification.



Source: Primary Data Processed, (2025)

Figure 5

Microscopic appearance of Meibomian gland duct diameter stained with Hematoxylin-Eosin (H&E) using a Nikon Eclipse Ei microscope at 400x magnification.

Polymorphonuclear Cell Infiltration

The mean polymorphonuclear (PMN) cell count in the cornea did not show significant differences among groups ($p = 0.201$). In contrast, PMN infiltration in the palpebral conjunctiva demonstrated a significant increase in the treatment groups compared with the control ($p = 0.014$).

This study demonstrated that topical administration of polyethylene nanoplastics induced inflammatory changes on the ocular surface of Wistar rats, particularly in the palpebral conjunctiva and Meibomian glands. A significant increase in PMN infiltration was observed in the conjunctiva, while corneal PMN counts showed no meaning-

ful difference between exposure groups and the control. This indicates that the conjunctiva may respond earlier and more prominently to nanoplastic exposure compared with the cornea, likely due to its higher vascularity, mucosal structure, and direct interaction with environmental particulates. PMN mobilization represents the primary acute inflammatory response, characterized by neutrophil recruitment, phagocytosis, and release of pro-inflammatory mediators such as IL-8, TNF- α , and ROS (Fortingo et al., 2022; Hanlon et al., 2014; Kobayashi, 2009; Livingston et al., 2019). The dose-dependent PMN elevation aligns with the hypothesis that nanoplastic particles can induce oxidative stress and trigger immune activation on the ocular surface (Das, 2023; Wang et al., 2025; Wu et al., 2023).

Meibomian Gland Histological Changes

The number of normal Meibomian gland acini was significantly reduced in exposed groups ($p = 0.000$), while no significant differences were observed in acinar cell diameter ($p = 0.453$) or Meibomian duct diameter ($p = 0.133$).

A reduction in the number of normal Meibomian acini in the exposed groups further suggests structural alteration of the glands. PE nanoplastics have been reported to induce tissue oxidative stress, inflammatory signaling, and cellular degeneration depending on concentration, exposure time, and particle size (Almeida et al., 2022; Jaeger et al., 2024; Palyama et al., 2023; Ragusa et al., 2021; Wu et al., 2023). In this study, although degeneration was evident histologically, acinar and ductal diameters showed no statistically significant change, indicating that early damage may begin at the cellular level before progressing to gross morphological obstruction. This finding supports the concept that Meibomian dysfunction develops gradually from acinar dropout and inflammatory infiltration toward later-stage duct dilation or obstruction, consistent with the notion of progressive meibomian gland pathology.

Post-hoc Analysis

Post-hoc LSD analysis for PMN in the palpebral conjunctiva showed significant differences between the control and X2 ($p = 0.003$) and X3 ($p = 0.014$), but not between control and X1 ($p = 0.197$). Inter-group comparison revealed that X1-X2 ($p = 0.051$) was near-significant, while X1-X3 ($p = 0.208$) and X2-X3 ($p = 0.403$) were not significantly different. Mann-Whitney analysis of normal Meibomian acini showed significant differences between control - X1 ($p = 0.004$) and control - X3 ($p = 0.003$), but not control - X2 ($p = 0.746$). Differences were also found in X1-X2 ($p = 0.004$) and X2-X3 ($p = 0.003$), while X1-X3 ($p = 0.564$) was not significant.

Post-hoc analysis provided additional clarity regarding the dose-response relationship. The LSD post-hoc test showed that significant PMN elevation in the palpebral conjunctiva occurred only at higher doses (X2 and X3), whereas X1 did not differ significantly from controls. This pattern suggests a threshold effect, where conjunctival inflammation becomes prominent only after exposure reaches at least 1 mg/0.1 cc/day. Conversely, the Mann-Whitney post-hoc findings for normal Meibomian acini revealed a non-linear pattern: X1 and X3 differed significantly from the control, while X2 did not. Significant differences between X1-X2 and X2-X3 further support a dose dependent but non-monotonic response. Such patterns are commonly observed in nanoparticle toxicology, where aggregation behavior, epithelial penetration variability, or uneven oxidative stress activation can produce irregular toxicological responses across dose levels. These findings imply that Meibomian acini may be more sensitive to early subclinical damage, whereas conjunctival inflammation requires stronger exposure to manifest significantly.

Interestingly, duct obstruction did not differ significantly between groups, which contrasts with reports showing particulate matter or air pollutants can induce ductal dilation and meibum flow disturbance (Tu et al., 2024). The absence

of significant obstruction in the current model may be influenced by shorter exposure duration, moderate dosing units, or polyethylene's relatively inert characteristics, suggesting that chronic exposure or higher concentrations may be required to produce more advanced glandular changes. The narrowing trend observed in duct diameter in visual data may represent an early adaptive response, possibly related to epithelial edema, altered lipid viscosity, or compensatory contraction of myoepithelial units.

The implications of these findings highlight that polyethylene nanoplastics despite being chemically stable possess the potential to induce chronic inflammatory reactions and early glandular degeneration. In clinical settings, persistent nanoplastic exposure could contribute to tear film instability, risk of dry eye development, and ocular surface irritation. This aligns with emerging evidence of micro- and nanoplastics detected in tear film samples and their association with ocular discomfort (Wang et al., 2025), demonstrating that this research model contributes valuable foundational insight in an area still lacking extensive in-vivo studies.

This study is limited by the relatively short exposure duration, absence of molecular or immunohistochemical analysis, and endpoint measurement at a single time point. The suspension was administered without homogenizing agents, potentially affecting particle distribution. The actual particle penetration into ocular tissue was also not quantified. Future research should explore longer exposure periods, wider dose ranges, tear biochemical markers, as well as molecular indicators of oxidative stress and apoptosis. A time-series model or imaging modalities such as meibography could further clarify disease progression trajectory.

Overall, this study suggests that topical polyethylene nanoplastic exposure can induce conjunctival inflammation and early Meibomian gland degeneration,

supporting the notion that nanoplastic particles pose potential ocular toxicity risk even in short-term models.

CONCLUSION

Based on this study, topical administration of polyethylene nanoplastic eye drops in Wistar rats resulted in a significant increase in polymorphonuclear (PMN) cells in the palpebral conjunctiva and a significant reduction in the number of normal Meibomian gland acini, indicating that nanoplastic exposure can induce ocular surface inflammation and contribute to early Meibomian gland alterations. In contrast, no significant differences were observed in corneal PMN count, acinar diameter, or Meibomian duct diameter, suggesting that short-term exposure did not produce measurable corneal infiltration or major morphological enlargement of Meibomian gland structures. These findings provide baseline evidence that polyethylene nanoplastics may pose potential risks to the ocular adnexa, particularly the conjunctiva and Meibomian glands. Further studies with longer exposure durations, larger sample sizes, and additional parameters including molecular inflammatory markers, oxidative stress profiles, tear film evaluation, and advanced imaging techniques are recommended to elucidate progressive glandular changes and long-term ocular effects of nanoplastic exposure.

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