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# Phagocytic Receptors Mediate Survival and Locomotor Resilience of Ethanol-Exposed Drosophila

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**Abstract**

**Background** Ethanol is a widely studied toxicant known to induce oxidative stress and cellular damage across species. While phagocytic clearance is essential for maintaining tissue homeostasis, its role in protecting against ethanol-induced toxicity remains poorly understood. This study aims to elucidate the role of phagocytic receptors in modulating the organism's response to ethanol-induced toxicity using Drosophila melanogaster.

**Methods** To assess the functional significance of phagocytic receptors, we utilized behavioral locomotor assay and survival analysis on both wild-type and mutants deficient in the phagocytic receptors Draper and Integrin-[beta]v of Drosophila which are homologous to mammalian MEGF10 and integrins, respectively. Flies were exposed to the various concentration of ethanol, and their climbing ability and survival responses were compared across genotypes.

**Results** Our results revealed that mutants lacking Draper and/or Integrin-[beta]v showed a significant reduction in locomotor activity ($p < 0.05$ to $p < 0.0001$) and an approximately two-fold decrease in survival time under ethanol exposure compared with wild-type flies. These findings indicate that impaired phagocytic clearance may exacerbate ethanol toxicity.

**Conclusion** In summary, this study demonstrates that phagocytic receptors play a critical protective role against ethanol toxicity in D. melanogaster. The data suggest the interconnected roles of oxidative stress, apoptosis, and phagocytosis in maintaining tissue homeostasis, validating Drosophila as a robust model for investigating the effect of toxicant on the phenotypic features of metazoan species.

**Keywords:** Drosophila; ethanol; toxicity; innate immunity; phagocytosis.

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## Introduction

The assessment of compound toxicity *in vivo* necessitates the use of an appropriate and reliable model organism to ensure accurate, reproducible, and mechanistically informative results (Parasuraman, 2011). *In vivo* model selection is critical, as it influences the translational relevance, cost, ethical considerations, and overall feasibility of a study (Doke & Dhawale, 2015). *Drosophila melanogaster* has gained widespread recognition as a powerful alternative model for toxicological research due to its low maintenance costs, minimal ethical concerns, short life cycle, high fecundity, and suitability for high-throughput screening (Abolaji et al., 2013; Nainu et al., 2022). Notably, *D. melanogaster* shares approximately 75% of disease-related genes with humans, enhancing its relevance in biomedical research (Pandey & Nichols, 2011). The availability of diverse mutant and transgenic lines further supports the

investigation of specific molecular pathways involved in toxicity (Khaerani et al., 2024; Pratama et al., 2025; Pratama et al., 2024; Troutwine et al., 2016).

Ethanol has been extensively studied in *D. melanogaster* as a model compound for investigating toxicological effects across multiple biological levels, ranging from behavior to molecular pathways (Sandhu et al., 2015; Tamar et al., 2024). In flies, ethanol impairs sleep, increases mortality (De Nobrega et al., 2022), and may influence tolerance and toxicity through circadian regulation and synaptic plasticity mechanisms (Peterson & Ahmad, 2024). At the cellular level, ethanol disrupts homeostasis by generating excessive reactive oxygen species (ROS), overwhelming antioxidant defenses, and causing tissue damage, as evidenced by decreased catalase activity in exposed flies (Padovan et al., 2023; Tamar et al., 2024).

Beyond the role of endogenous antioxidants in neutralizing reactive oxygen species (ROS), the body also engages apoptosis, known as a programmed cell death, as a key physiological response to elevated cellular stress (Redza-Dutordoir & Averill-Bates, 2016). However, the apoptosis process and restoration of tissue homeostasis depend critically on the efficiency of the phagocytic system, which is responsible for the recognition and clearance of apoptotic bodies (Arandjelovic & Ravichandran, 2015). Disruption in this process may exacerbate cellular stress and contribute to ethanol-induced toxicity, underscoring the complex interplay between oxidative stress, programmed cell death, and immune-mediated clearance mechanisms.

The interplay between phagocytosis and apoptosis is a highly conserved biological process observed from mammals to *D. melanogaster* (Bangs et al., 2000; Nainu et al., 2017). In *Drosophila*, two primary receptors have been reported to mediate the phagocytic clearance of apoptotic cells: Draper (encoded by *drpr*) and Integrin-β*v* (encoded by *itgbn*) (Nagaosa et al., 2011; Shiratsuchi et al., 2012; Tung et al., 2013; Zheng et al., 2017). These receptors have been shown to perform functions analogous to their mammalian counterparts, MEGF10 and integrin, respectively (Melcarne et al., 2019). Their evolutionary conservation underscores a fundamental role in maintaining tissue homeostasis through the efficient recognition and removal of apoptotic cells.

This study specifically investigates how the phagocytic receptors Draper and Integrin-β*v* contribute to ethanol-induced toxicity in *Drosophila*, providing new insight into the role of phagocytosis in toxicant resilience. Utilizing *Drosophila* as a model offers a strategic and efficient platform to explore biological processes underlying ethanol toxicity that would be more challenging to study in more complex animal models.

## Method

*Drosophila* stock

This study utilized adult male and female $w^{1118}$, $itgbn^{2}$, and $itgbn^{2};drpr^{\Delta 5}$ *Drosophila* strain (provided by the Laboratory of Host Defense and Responses at Kanazawa University). The flies were bred and maintained in culture vials containing corn-meal based fly food and were kept at 25°C, 12 hours light and 12 hours dark cycle.

Ethanol preparation

A range of ethanol solutions was prepared by diluting a 96% ethanol stock with water to obtain final concentrations of 65%, 45%, and 25% of ethanol.

Study group and experimental design

In this study, *D. melanogaster* from three different genotypes were utilized. The control group consisted of the $w^{1118}$ strain, which possesses intact phagocytic receptor function. The experimental groups included the $itgbn^{2}$ single mutant (deficient in the Integrin-β*v* receptor) and the $itgbn^{2};\ drpr^{\Delta 5}$ double mutant (lacking for functional Draper and Integrin-β*v* receptors).

For each genotype, 10 flies were tested per replicate, with experiments repeated in at least three biological replicates. Male and female flies were assessed separately to examine sex-dependent responses. Each group was exposed to 1.000 µL of ethanol at different concentrations by applying the solution to the top of the vial plug, which was then sealed with an additional plug (Sandhu et al., 2015). After ethanol exposure, locomotor and survival assays, were performed.

In the locomotor assay, flies were placed in a pre-marked, empty vial. The vial was tapped three times to ensure all flies settled at the bottom, and their climbing activity was observed for 15 seconds. The number of flies that crossed the marked line during this period was recorded as a measure of locomotor ability (As'ad et al., 2023).

The survival assay was conducted by tracking the number of flies that remained alive over a 60-minute period following ethanol exposure. Throughout the observation phase, each vial was gently tapped three times every 10 minutes, and the number of surviving flies was recorded accordingly (Tamar et al., 2024).


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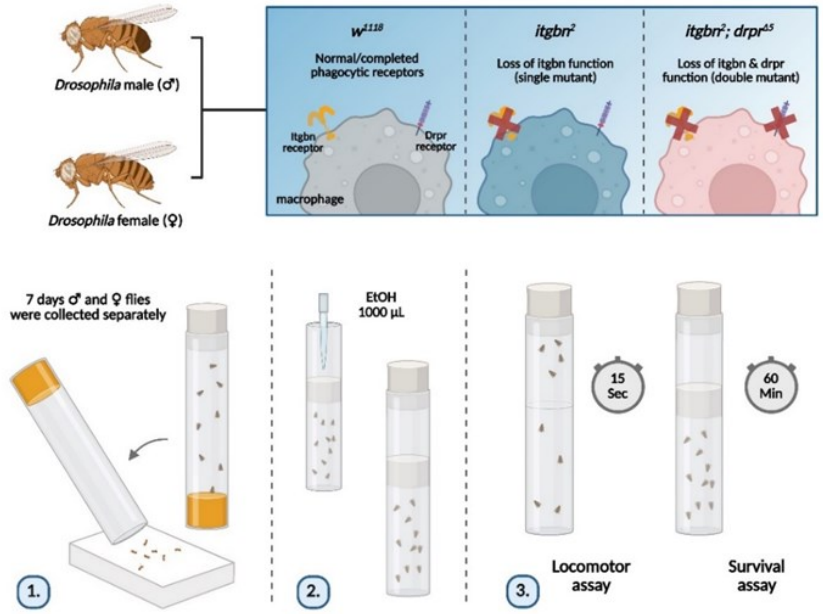


**Figure 1.** Study group and experimental design. Seven-day-old male and female of w1118, itgbn2, and itgbn2; drprΔ5 flies were separated, exposed to 1000 µL ethanol, and were assessed by climbing assay (15 s) and survival analysis (60 min).

Data processing and analysis

Results obtained from at least three independent biological replicates were processed using GraphPad Prism® version 9. Data obtained from the locomotor assay were presented as bar graphs and were analyzed using a Two-Way ANOVA. In contrast, survival data were analyzed and presented based on the Kaplan-Meier method, with statistical significance assessed via the Log-Rank test. All results are expressed as mean ± standard deviation (SD), and a p-value < 0.05 was considered statistically significant.

## Result and Discussion

Utilizing simple model organisms like *D. melanogaster* facilitates the exploration of specific physiological pathways that may be influenced by chemical exposure. In this study, we investigated the impact of phagocytic receptor function on the response to ethanol-induced toxicity in *Drosophila*. As an invertebrate model, *Drosophila* offers significant advantages due to its well-characterized immune system (Buchon et al., 2014) and the availability of diverse genetic mutants. These features allow precise manipulation and investigation of specific immune components. In this study, we focused on two key phagocytic receptors, Draper and Integrin-β*v*, to assess their roles in mediating the toxic effects of ethanol.

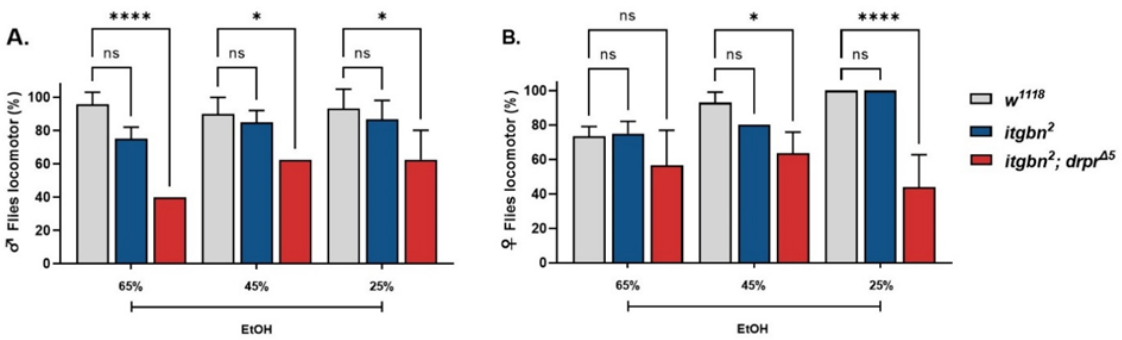


**Figure 2.** Locomotor ability of male (A) and female flies (B) after ethanol exposure. A significant reduction in the locomotor activity was observed specifically in the double mutant group, whereas the single mutant showed no notable difference. Each group was compared to the control ($w^{1118}$) flies. ns, non-significant; * p < 0.05; ****p<0.0001.


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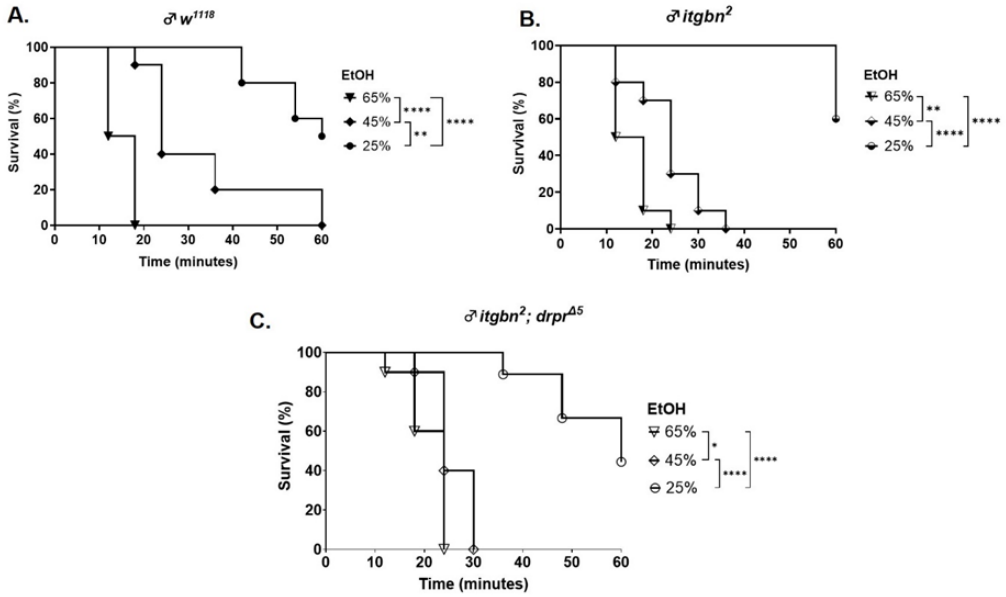


**Figure 3.** Survival rate of male $w^{1118}$ (A), *itgbn*$^{2}$ (B) and *itgbn*$^{2}$*; drpr*$^{\Delta 5}$ flies after ethanol exposure. The data was recorded in 60 minutes. * $p < 0.05$; **$p<0.01$; ****$p<0.0001$.

At the initial stage of the study, we evaluated the locomotor behavior of both male and female flies following exposure to different ethanol concentrations, aiming to investigate the possibility of gender-specific responses. The

findings (Figure 2A-B) showed that flies with a single mutation in itgbn² did not exhibit significant differences in locomotor activity compared to the w1118 control. Notably, double mutant flies lacking for both itgbn² and drprΔ5 displayed a substantial decline in locomotor ability ($p < 0.05$ to $p < 0.0001$). The decline in locomotor performance became increasingly evident with higher ethanol concentrations, suggesting a dose-dependent vulnerability associated with impaired phagocytic receptor function.

These findings suggest that phagocytic receptors play a critical role in maintaining physiological homeostasis, particularly in responding to the cellular stress induced by ethanol exposure. The absence of significant differences between the *itgbn²* mutant and the $w^{1118}$ control indicates that Draper may compensate for the loss of Integrin-β*ν*, allowing the flies to effectively mitigate ethanol-induced stress. However, in the double mutant lacking for both Integrin-β*ν* and Draper, this compensatory mechanism is disrupted, leading to physiological imbalance. This disruption may result from increased cellular stress and death that cannot be efficiently cleared, ultimately contributing to a decline in energy availability for locomotor activity and a marked impairment in behavioral performance (Nguyen et al., 2023; Vakifahmetoglu-Norberg et al., 2017). Additionally, both male and female flies displayed comparable response patterns at ethanol concentrations of 45% and 25%, suggesting no significant sex-based differences in behavioral sensitivity under these conditions. Therefore, subsequent experiments focused on male flies from each genotype to further investigate the effects of ethanol exposure on survival outcomes.

The survival assay indicated a concentration-dependent effect, with higher ethanol concentrations leading to progressively faster onset of mortality and reduced survival rates across all genotypes. Loss of receptor function accelerated mortality, supporting a model in which insufficient removal of apoptotic cells leads to secondary tissue damage and systemic decline (Strzyz, 2017). Recent mammalian studies show that integrins regulate neutrophil phagocytosis, and macrophage efferocytosis is central to tissue homeostasis (Pulikkot et al., 2022; Sheng et al., 2024). Ethanol further increases apoptotic burden and activates autophagy–phagocytosis pathways in macrophages (Betsuyaku et al., 2024) suggesting that impaired clearance is a conserved driver of ethanol-induced tissue damage across species.

This mechanism is likely conserved, as mammalian orthologs of Draper (MEGF10) and integrins also regulate apoptotic cell clearance and tissue integrity. Accordingly, our findings point to a fundamental protective pathway with potential relevance for understanding alcohol-induced pathologies in higher organisms.

These findings underscore that efficient phagocytic activity is essential not only for immune defense but also for limiting tissue damage under toxic stress, a principle relevant

to human alcohol-related injury. While our study highlights behavioral and survival outcomes, future work incorporating molecular markers of oxidative stress and apoptosis, as well as additional receptors, will be important to define the mechanisms and networks that sustain resilience against chemical stressors.

## Conclusion

This study demonstrates the essential role of phagocytic receptors Draper and Integrin-βν in modulating the physiological response to ethanol-induced toxicity in *D. melanogaster*. Loss of phagocytic function, particularly in the absence of both receptors, results in impaired locomotor activity and accelerated mortality, likely due to the accumulation of uncleared apoptotic cells and subsequent secondary necrotic damage. These results underscore the importance of phagocytosis in maintaining cellular homeostasis under toxic stress and establish *Drosophila* as a valuable model for exploring immune-related mechanisms of toxicological responses. Future work should investigate the downstream signaling pathways of these receptors and assess whether therapeutic modulation of phagocytic clearance can mitigate alcohol-related tissue injury.

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