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ORIGINAL ARTICLE

Impact of Orlistat on *Staphylococcus aureus* Engulfment by Phagocytic Cells In Vitro

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ABSTRACT

Background: *In recent years, anti-obesity drugs have become increasingly widespread. With their growing use, it is essential to study the effects of these medications on vital physiological processes to better understand both their efficacy and potential adverse effects. One such crucial process is phagocytosis, a key mechanism in the immune response.* ***Objective:*** *This study aimed to evaluate the effects of the anti-obesity drug Orlistat on phagocytic activity in vitro.* ***Methodology:*** *The Phagocytic function was assessed using a Phagocytic Function Test, where phagocytic cells were exposed to various dilutions and concentrations of Orlistat. The cells were incubated and examined at two different time intervals: 30 minutes and 1 hour.* ***Results:*** *The results showed no direct effect and no statistically significant difference in phagocytic activity at any of the tested concentrations after 30 minutes and 1 hour of incubation. However, a significant difference ($p < 0.05$) was observed when comparing results between the first and third experimental slides.* ***Conclusion:*** *Although Orlistat did not demonstrate a significant impact on phagocytic activity during the initial 30 to 60 minutes of exposure, the differences observed between specific samples suggest that extended exposure or experimental variability might influence phagocytic responses. Further research with longer exposure times and more controlled conditions is warranted to clarify these findings.*

INTRODUCTION

Orlistat (Tetrahydrolipstatin) functions as an irreversible inhibitor of lipase enzymes. It is chemically derived from the natural compound lipstatin, which is produced by the microorganism *Streptomyces toxytricini*[1]. Orlistat is recognized for its safety and efficacy in inhibiting both pancreatic and gastric lipases within the gastrointestinal tract and has been approved by the U.S. Food and Drug Administration (FDA) for the treatment of obesity[2]. Structurally, orlistat is the hydrogenated form of lipstatin, a potent natural lipase inhibitor extracted from *Streptomyces toxytricini*[3-4].
Beyond its primary use in weight management, orlistat has been shown to induce a modest reduction in blood pressure and may delay the onset of type 2 diabetes. These benefits are thought to result either from weight loss or additional, yet to be fully understood, mechanisms[5-6]. Despite its therapeutic advantages, orlistat is frequently associated with gastrointestinal side effects—often referred to as "treatment effects"—such as steatorrhea, characterized by oily and loose stools. These adverse effects typically decrease over time and are the most commonly reported with orlistat use[7].

METHODOLOGY

Bacterial Suspension Preparation

To prepare the bacterial suspension for the **Engulfment Function Test**, a few *Staphylococcus aureus* isolates were placed in 5 ml of sterile normal saline and mixed thoroughly.

Orlistat Dilution Preparation

Three dilutions of Orlistat were prepared as follows:

- Dilution 1: 4 mg/ml
- Dilution 2: 2 mg/ml
- Dilution 3: 1 mg/ml

Phagocytosis (Ingestion) Test

A heparinized capillary tube was filled up to two-thirds of its length with blood. A drop of the prepared bacterial suspension was placed on a sterile glass slide. Heparinized blood was mixed with the bacterial suspension on the slide. The capillary tube was refilled with the blood-bacterial suspension mixture. The capillary tube was incubated horizontally at 37ºC for 30 minutes, with gentle mixing every 5 minutes. After incubation, the capillary tube was centrifuged for 2 minutes using a hematocrit centrifuge at 10,000 rpm. A smear was prepared from the buffy coat on a clean glass

slide. The smear was air-dried, fixed with a few drops of absolute methanol, stained with Leishman's stain, and examined under a light microscope using an oil immersion lens.

Calculations:

- **Percentage of neutrophils ingesting bacteria (N+%):** The percentage of neutrophils containing ingested bacteria.
- **Average number of bacteria per positive neutrophil:**
- Average bacteria per positive neutrophil (B/N+) = Total number of bacteria (B) / Total number of positive neutrophils (N+)[8]

Leishman`s stain:

The prepared smear was first air-dried, then the slide was completely covered with the staining solution. After an initial staining period of two minutes, twice the volume of water was added to the slide, allowing the staining to continue for an additional 5 to 7 minutes. The slide was then gently rinsed under a stream of buffered water (pH 7.2) until a faint pink coloration appeared, which typically took up to two minutes. After cleaning the reverse side of the slide, it was placed vertically and left to air-dry completely[9-10].

Statistical Analysis

All data were statistically analyzed using **SPSS version 12**. The **t-test (two-tailed)** and **ANOVA** tests were applied to assess differences between groups.

RESULTS

Table 1 shows that across all dilutions, there were **no statistically significant differences** (**p > 0.05**) compared to the control at 30 minutes, except for the comparison between dilution 1 and dilution 3 (**p = 0.001**), which showed a significant difference.

Table 2 shows that all dilutions had no statistically significant differences (**p > 0.05**) compared to the control at 30 minutes, except for the comparison between control 1 and dilution 2, which showed a significant difference (**p = 0.041**).

Table 1: The phagocytic activity of neutrophils among different dilutions of drug in 30mint.

Number of samples = (10)			
N.O	**Parameter**	**Mean ± SE**	**P – Value**
1	Control – 30min	4.90 ± 1.120	0.345
	Dilution 1– 30min	3.70 ± .423	
2	Control – 30min	4.90 ± 1.120	0.535
	Dilution 2– 30min	6.10 ± .948	
3	Control – 30min	4.90 ± 1.120	0.405
	Dilution 3– 30min	6.10 ± .737	
4	Dilution 1– 30min	3.70 ± .423	0.57
	Dilution 2– 30min	6.10 ± .948	
5	Dilution 1– 30min	3.70 ± .423	.001 **
	Dilution 3– 30min	6.10 ± .737	
6	Dilution 2– 30min	6.10 ± .948	1.00
	Dilution 3– 30min	6.10 ± .737	

Table 2: The phagocytic activity of neutrophil among different delusion of drug in one hour.

Number of samples = (10)			
N.O	**Parameter**	**Mean ± SE**	**P – Value**
1	Control – 1 Hour	7.70 ± 1.044	0.151
	Dilution 1– 1 Hour	11.30 ± 1.752	
2	Control – 1 Hour	7.70 ± 1.044	0.041*
	Dilution 2– 1 Hour	12.60 ± 3.133	
3	Control – 1 Hour	7.70 ± 1.044	0.382
	Dilution 3– 1 Hour	14.20 ± 2.356	
4	Dilution 1– 1 Hour	11.30 ± 1.752	0.311
	Dilution 2– 1 Hour	12.60 ± 3.133	
5	Dilution 1– 1 Hour	11.30 ± 1.752	0.281
	Dilution 3– 1 Hour	14.20 ± 2.356	
6	Dilution 2– 1 Hour	12.60 ± 3.133	0.214
	Dilution 3– 1 Hour	14.20 ± 2.356	

Table 3 compares the dilutions across the two time points. Most comparisons showed statistically significant increases in phagocytic activity over time, particularly between 30 minutes and 1 hour for: Dilution 1 ($p = 0.001$), Dilution 2 ($p = 0.009$ and $p = 0.001$), and Dilution 3 ($p = 0.023$ and $p = 0.011$). However, some comparisons, such as those involving Dilution 3 at 30 minutes versus other dilutions at 1 hour, did not reach statistical significance difference ($p > 0.05$).

Table 3: The phagocytic activity of neutrophil among different dilution of drug between 30mint and one hour.

Number of samples = (10)			
N.O	**Parameter**	**Mean ± SE**	**P – Value**
1	Dilution 1– 30min	3.70 ± .423	**0.001***
	Dilution 1– 1 Hour	11.30 ± 1.752	
2	Dilution 1– 30min	3.70 ± .423	**0.009***
	Dilution 2– 1 Hour	12.60 ± 3.133	
3	Dilution 1– 30min	3.70 ± .423	**0.023***
	Dilution 3– 1 Hour	11.30 ± 1.752	
4	Dilution 2– 30min	6.10 ± .948	0.419
	Dilution 1– 1 Hour	11.30 ± 1.752	
5	Dilution 2– 30min	6.10 ± .948	**0.001***
	Dilution 2– 1 Hour	12.60 ± 3.133	
6	Dilution 2– 30min	6.10 ± .948	**0.000****
	Dilution 3– 1 Hour	14.20 ± 2.356	
7	Dilution 3– 30min	6.10 ± .737	0.328
	Dilution 1– 1 Hour	11.30 ± 1.752	
8	Dilution 3– 30min	6.10 ± .737	**0.011***
	Dilution 2– 1 Hour	12.60 ± 3.133	
9	Dilution 3– 30min	6.10 ± .737	0.601
	Dilution 3– 1 Hour	14.20 ± 2.356	

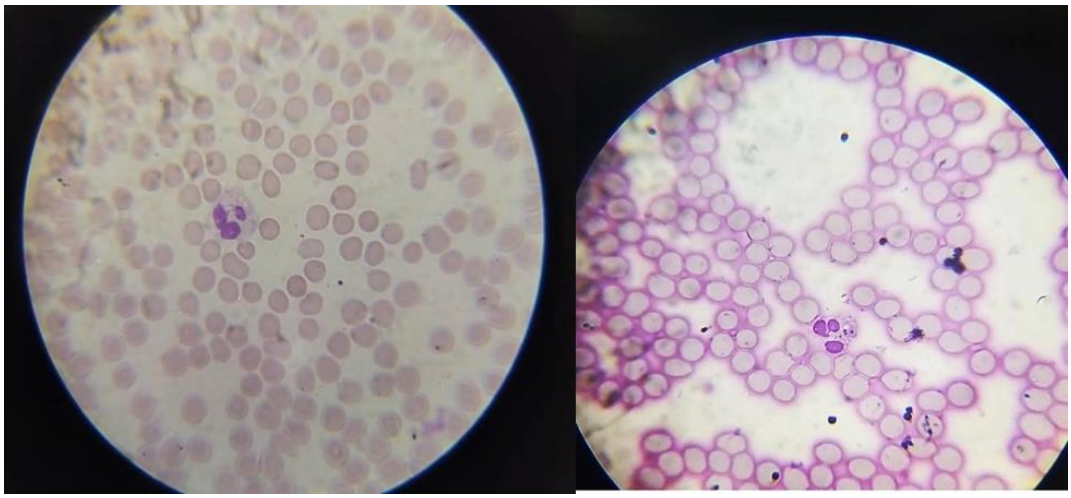

Fig. 1: Shows bacteria engulfed inside a phagocytic cell.

This study investigated the immunomodulatory effects of Orlistat—an anti-obesity agent—by evaluating its influence on neutrophil activity, a key component of innate immune function. The results revealed time- and concentration-dependent changes, suggesting a delayed but measurable impact on neutrophil-mediated immunity. At 30 minutes, no significant differences in neutrophil activity were observed compared to the control group, except for a statistically significant increase between dilution 1 and dilution 3.

DISCUSSION

This suggests that Orlistat does not exert an immediate immunomodulatory effect but may begin to slightly affect neutrophil function at higher concentrations. These findings align with previous studies showing that many pharmacological agents require time for absorption, uptake, or metabolism before modulating immune pathways[11-12].

After one hour, a marked increase in phagocytic activity was observed across all dilutions, with statistically significant enhancement particularly noted at dilution 2. This temporal activation supports previous research suggesting that Orlistat may influence lipid metabolism in ways that impact immune signaling, especially in immune cells such as macrophages and neutrophils, which are sensitive to fluctuations in fatty acid levels and membrane lipid composition[13-14]. The most pronounced effects were seen at higher concentrations (dilutions 2 and 3), indicating a dose-dependent enhancement of innate immune function. These findings imply that Orlistat may possess secondary immunomodulatory properties beyond its established metabolic role—an important consideration given that obesity is frequently associated with chronic low-grade inflammation and impaired immune responses[15].

Macrophages, key phagocytic cells of the innate immune system[16-17], express high levels of lipoprotein lipase (LPL). Orlistat's primary mechanism—inhibition of intestinal lipases—modifies lipid availability and uptake, which may influence membrane fluidity, receptor-mediated phagocytosis, and downstream intracellular signaling in neutrophils[18]. Moreover, Orlistat, as an LPL inhibitor, significantly reduces triglyceride uptake from very low-density lipoproteins (VLDL) by macrophages such as THP-1 cells[19]. This confirms that LPL inhibition by Orlistat can alter macrophage lipid metabolism. Fatty acid metabolism is intricately linked to immune cell activation, and disruptions in lipid pathways can affect phagocytic capabilities[20-21].

Further evidence suggests that Orlistat may modulate macrophage function beyond lipid metabolism. For instance, Orlistat has been shown to reverse arsenite-induced inhibition of macrophage activity by upregulating ABCA1 expression, thereby enhancing intracellular arsenite clearance[22]. While the precise interaction between Orlistat and LPL in macrophages remains to be fully elucidated, these findings establish a mechanistic link between Orlistat, lipid regulation, and immune function.

Additionally, Orlistat may exert anti-tumor and immunomodulatory effects through inhibition of fatty acid synthase (FASN), an enzyme involved in lipogenesis and immune cell differentiation. Inhibiting FASN may promote the polarization of myeloid precursors toward pro-inflammatory M1 macrophages, contributing to innate immune activation[23-24]. However, this immunostimulatory effect is context- and concentration-dependent. Select mitogens may trigger FASN inhibition, leading to enhanced phagocytosis, while in other contexts, FASN suppression is associated with anti-inflammatory effects[25-26].

The findings demonstrate that Orlistat does not produce an immediate immunomodulatory effect (no differences observed compared to control at 30 minutes), aligning with the necessity for pharmacological agents to allow adequate time for absorption or metabolism to initiate their biological pathways[11-12].

The statistically substantial increase in phagocytic activity after one hour, especially at Dilution 2, strongly suggests that Orlistat improves the function of the innate immune system. This is consistent with Orlistat's recognized action as an LPL inhibitor[19-22], given that alterations in lipid metabolism intricately influence cell membrane stability and the phagocytic capabilities of immune cells, including neutrophils and macrophages[13-18-20].

This impact is thought to be due to an indirect process that has to do with how lipids are broken down. This conclusion is very important because obesity is commonly linked to a weakened immune system[15-26].

Although the enhanced neutrophil function observed in this study may indicate improved innate immune surveillance—potentially beneficial in obese individuals—these findings should be interpreted with caution. While moderate activation of macrophages and neutrophils may support immune function, excessive or prolonged stimulation could exacerbate pro-inflammatory conditions, particularly in individuals with metabolic syndrome, where immune hyperactivity is already a concern[26-27].

CONCLUSION

Although Orlistat did not demonstrate a significant impact on phagocytic activity during the initial 30 to 60 minutes of exposure, the differences observed between specific samples suggest that extended exposure or experimental variability might influence phagocytic responses. Further research with longer exposure times and more controlled conditions is warranted to clarify these findings.

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Data availability
All clinical and statistical data and materials are available for the benefit of science.

Code availability Not applicable.

Declarations

Conflict of interest
The author declare that they have no conflicts of interest.

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