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Impact of *Aspergillus spp*. lipase to Influence Histological and Biochemical Deviations in Female Rats

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Rats,
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Histological.

ABSTRACT
Fungal lipases, including those produced by *Aspergillus niger*, are widely used in industrial, pharmaceutical, and food applications due to their catalytic efficiency, yet their potential toxicological effects on biological systems remain poorly understood. Given increasing exposure to these enzymes, evaluating their biochemical and histopathological consequences is essential for assessing safety risks. This paper analyses the biochemical and histological impacts of lipase from *Aspergillus niger* in female albino rats under laboratory conditions. Twenty adult female rats were separated into control and treated groups. The treatment groups received intraperitoneal injections of lipase at two concentrations over a three-week period. Biochemical parameters were assessed by measuring interleukins (IL-1α, IL-2, IL-6, and IL-8) and oxidative stress indicators malondialdehyde (MDA) and glutathione (GSH). Liver tissues were examined histologically to identify structural changes. Findings revealed that interleukin levels in treated groups increased significantly, indicating activation of inflammatory processes. Elevated MDA reflected increased lipid peroxidation, while altered GSH levels indicated impaired antioxidant defense systems, together affirming oxidative stress caused by lipase exposure. Histological examination showed structural liver damage, including hepatocellular degeneration, central vein wall thickening, and inflammatory cell infiltration, with more severe changes in the higher-dose group, indicating a dose-dependent effect. In conclusion, female rats exposed to *Aspergillus niger* lipase experienced significant biochemical imbalances and liver tissue damage. This study identifies fungal lipases as inducers of oxidative stress and inflammation contributing to enzyme-induced toxicity, providing a basis for further investigation into their role in disease development and toxicological mechanisms.

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INTRODUCTION

An aspergillum, plural aspergilla, aspergillums, or aspergills, is a sprinkling brush or a container with holes punched into it used in Roman Catholic liturgy. Aspergillus, plural aspergilli, or simply "Aspergilli" in common use, is a generic name for a wide range of molds in mycology that reproduce asexually by producing spores on a body shape similar to a holy water fountain. The name

of the genus was first given by Antonio Micheli (1679–1736) in his 1727 pamphlet Nova plantarum, which included 1900 species of plants, 1400 of which were new species, and 900 of them were fungus (Ainsworth, 1976; Bennett, 2009). According to Balajee et al. (2007), aspergilli may cause a variety of diseases, including those that present on the skin, infections of the ears (otomycosis), and infections that spread deeper into the body (pulmonary aspergillosis and endocarditis). When it comes to Aspergillus Niger, morphology-based identification methods are crucial for clinical microbiology labs. The awareness of sexual or asexual structures, as well as their size, color, ornamentation, form, and attachment method, are diagnostic criteria (Pagano et al., 2007). While A. flavus and A. Niger are the most common, A. parasiticus, A. ochraceus, A. carbonarius, and A. alliaceous are also common. At several stages, including pre-harvest, harvest, processing, and management, they may contaminate agricultural goods. (Perrone et al., 2007). Hydrolysis of glycerophospholipids may be facilitated by a diverse family of enzymes known as phospholipases (Djordjevic, 2010). Base on their ability to cleave ester linkages within phospholipid molecules, lipases are classified into four main types: lipase A (LA1 and LA2), lipase B (LB), lipase C (LC), and lipase D (LD) (Richmond & Smith, 2011). While LC and LD are polypides that contribute to the phosphodiesterase class, LA and LB are philinids that belong to the acyl hydrolase group (Jasim & Saleh, 2020). Fungal enzymes have gained significant interest in the biomedical research since they can modify the metabolic pathways and cellular integrity of the hosts. Lipases of fungal origin are of particular interest among them since they are directly related to lipid constituents of biological systems. Lipids are vital components of cell membranes and they are vital in ensuring stability of the structure, signals, and storage of energy. Any alteration in lipid metabolism may, thus, be highly significant in the cellular homeostasis. The activity of fungal lipases with the host tissues can cause oxidative stress, which is one of the major mechanisms of cellular damages (Huang et al., 2019). Lipid peroxidation and damage of proteins and nucleic acids may be caused by the breakdown of lipids, which can produce reactive oxygen species (ROS). Such oxidative imbalance has the potential to deteriorate normal cellular functions and initiate inflammatory reactions, further amplifying tissue damage (Huang et al., 2019).

Bio-chemical biomarkers are often used in experimental studies as a surrogate of enzyme-induced toxicity. These biomarkers present the information concerning physiological disruptions on an overview level. Indicatively, an imbalance of lipid metabolism can cause a change in the level of cholesterol and triglycerides, and oxidative stress can affect the activities of antioxidant enzymes, including catalase and superoxide dismutase. These biochemical markers are vital towards the interpretation of how much metabolic disturbance has been brought about by enzymatic exposure (El-Korany et al., 2020). At the tissue level, enzymatic stress can lead to structural modifications that are not always immediately apparent through biochemical analysis alone. Microscopic evaluation of organs helps in identifying subtle and progressive changes such as cellular swelling, vascular congestion, and disruption of normal tissue organization. These histological observations are critical for correlating functional impairment with structural damage. One more important consideration is the role of dose and duration of exposure in determining the severity of enzymatic effects. Short-term exposure may result in reversible cellular changes, whereas prolonged exposure can lead to irreversible damage and organ dysfunction. Therefore, controlled experimental designs are necessary to evaluate both acute and chronic effects of enzymatic activity. Sex-based biological differences can influence the response to external agents, including enzymes. Female experimental models may exhibit distinct metabolic and hormonal responses, which can affect the degree of biochemical and histological alterations. This makes it important to specifically evaluate enzyme effects in female subjects to obtain more comprehensive and applicable results. Investigating the effects of fungal lipases under controlled laboratory conditions provides valuable insight into their potential role in disease progression and toxicity. Such studies not only enhance understanding of enzyme-mediated cellular damage but also contribute to the development of strategies for preventing or minimizing adverse biological effects (Durr-e-Nayab et al., 2018; Kulas et al., 2019).

Previous experimental investigations have highlighted the significant role of fungal-derived enzymes in inducing metabolic and structural alterations in biological systems. Several studies have reported that lipases produced by fungal species are capable of disrupting lipid metabolism and promoting oxidative imbalance within tissues (Alabdalall et al., 2020). According to available literature, exposure to fungal lipases has been associated with increased lipid peroxidation and reduced antioxidant defense mechanisms, which may lead to cellular injury and functional impairment of vital organs. Other investigators have found that there are structural changes in the vital organs after an exposure to fungal enzymes. Hepatocellular degeneration, tubular damage in the kidneys, inflammatory cell infiltration and vascular congestion are some of the common changes. These tissue-level changes are also usually accompanied by concordant biochemical observations such as high differences in serum enzyme and indicators of metabolism. Besides, fungi can modulate normal physiological activities by affecting the biochemical parameters like liver and renal physiological markers with

experimental research designs conducted using rat models, where fungal enzymes and metabolites were shown to disrupt physiological functions in normal systems (Ohnishi, Yoshida, & Sekiguchi, 1994) . These papers focus on the value of controlled laboratory studies that would enhance comprehension of the pathological events that fungal lipases cause. Thus, further studies are required to extend the investigations on the histological and biochemical deviations linked with Aspergillus spp. lipase exposure, especially in female experimental models.

MATERIALS AND METHODS

Animal model

Twenty adult female albino rats ranging in weight from 200 to 250 g and months of age from 5 to 8 were procured from the Technical College at North Technical University for the purpose of this investigation. The experiment had a pre-experiment period of two weeks of acclimatization of the rats under standard laboratory conditions and a normal pellet diet with unrestricted access to water to assure the normal physiological condition and the exclusion of any infections. In order to limit the stress on the animals that might confound the biochemical and histological measurements, the animals were kept in well-ventilated cages at controlled feather of thermal and light levels (12 h light/dark cycle). The specific rats used were females to assess sex specific metabolism and hormonal response levels to exposure to Aspergillus spp. lipase because they could determine exposure to enzymatic toxicity. All the laboratory operations were conducted in an ethically correct way and with institutional endorsement of such animal research. This animal model subjected to controlled conditions allowed the accurate following of biochemical indicators and histological alteration occasioned by the fungal lipase providing reliable and reproducible outcomes (Ohnishi et al., 1994).

Aspergillus Niger Isolates

The University of Baghdad College of Sciences Department of Biology provided these Aspergillus Niger isolates. They were separated from beans and linen. All fungal isolates were cultured on potato dextrose agar (PDA) and kept at 4˚C (Liu et al., 2016).

Lipase Detection

The technique of Durr-e-Nayab et al. (2018) identified lipase-containing media.

Lipase Etraction

Lipase from Aspergillus Niger was extracted using method (Memon et al., 1983).

Partial Purification of Enzyme

An enzyme extract was centrifuged at 6,000 rpm for 30 minutes on a magnetic stirrer with 60% NH4SO4. A 0.01 sodium citrate buffer solution at pH 6.7 dissolved the deposit. The material was then stored at -20 °C until needed.

Experimental Design

Male rats were used and divided to three groups as follow (each group consist six rats):

1. Control: As a control group, rats were given a standard diet.
2. Second group: After 3 weeks of intraperitoneal lipase enzyme injection (0.05ml/day per rat), animals were killed.
3. Third group: injected (intraperitoneally) with (0.1ml/day per rat) lipase for 3 weeks and then killed.

Measurements

Detection of Interleukins

According to BioSource Company's manufacturer's instructions, the ELISA technique was used to detect interleukin. All research firms have detected interleukin concentrations after two and four weeks following infections.

Oxidative stress factors

MDA (malondialdehyde), by Thio barbituric acid (TBA) according to method (Mahmood, 2010), and Glutathione (GSH) by using DTNB, with estimate catalase according to method (BM & Mahmood, 2013).

Samples collection

Blood samples were placed in test containers after being drawn under anesthesia via a cardiac puncture. Blood sample tubes were spun at 5000 rpm for 10 minutes after clotting to extract serum. Quickly after removal, the lung was homogenized with NaCl. The remaining serum and supernatant were then refrigerated for later use.

Statistical analysis

The significance of variability between the treatment and control groups was determined by analyzing the data using Minitab and the Analysis of Variance (ANOVA) test.

RESULTS AND DISCUSSION

Interleukin concentrations

"IL-1α, IL-2, IL-6 and IL-8 in rats of second and third groups that injected with Aspergillus Niger lipase show significant increased ($P<0.05$) compared with control rats as shown in figures" (1-4).

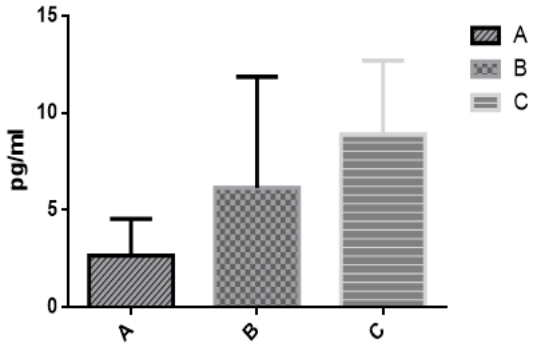

Figure 1: Levels of IL-1α in all groups

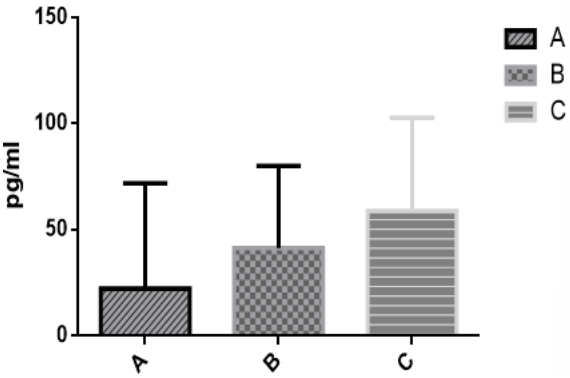

Figure 2: Levels of IL-2 in all groups

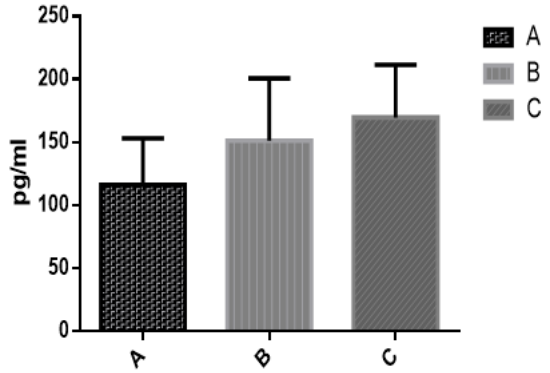

Figure 3: Levels of IL-6 in all groups

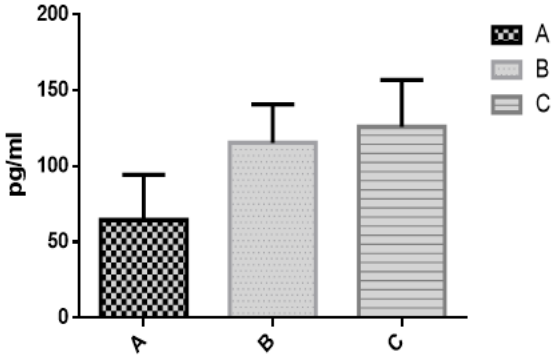

Figure 4: Levels of IL-8 in all groups

MDA and GSH Level

Figures 5-6 reveal that, in comparison to control rats, animals injected with Aspergillus Niger lipase in the second and third groups had significantly higher levels of MDA and GSH ($P<0.05$).

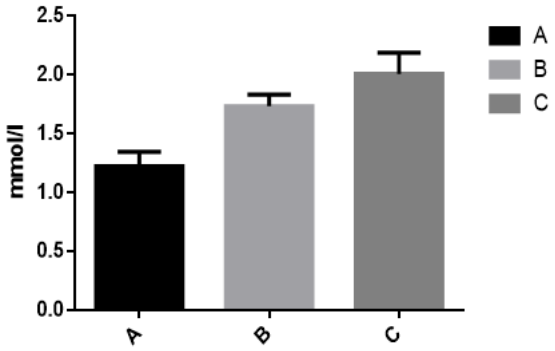

Figure 5: Level of MDA in all groups

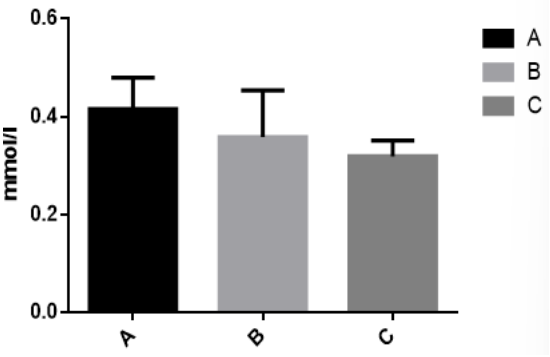

Figure 6: Levels of GSH in all groups

Histological study

Hepatocytes are arranged in a radical fashion around the principal veins in the liver sections taken from the control group, indicating a normal structural shape. Figure 7 further shows that the sinusoids' dimensions, which are situated between hepatocytes, are normal (7).

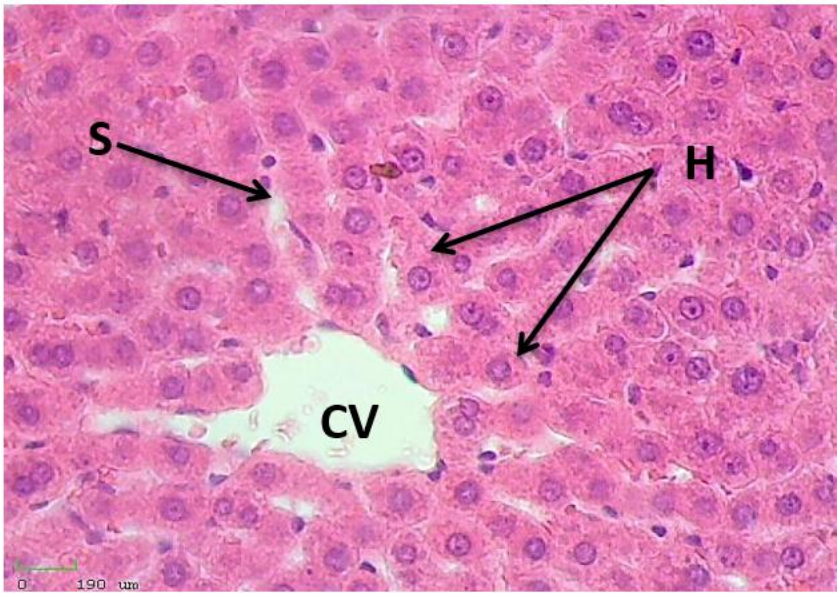

Figure 7: Liver of control group shows normal of central vein (CV), hepatocytes (HC) and sinusoids (S) H&E X400

Hepatocyte degradation, central venous wall thickening, and mono-nucleated inflammatory cell infiltration are seen in liver sections cut from the second and third groups (pictures 8-9).

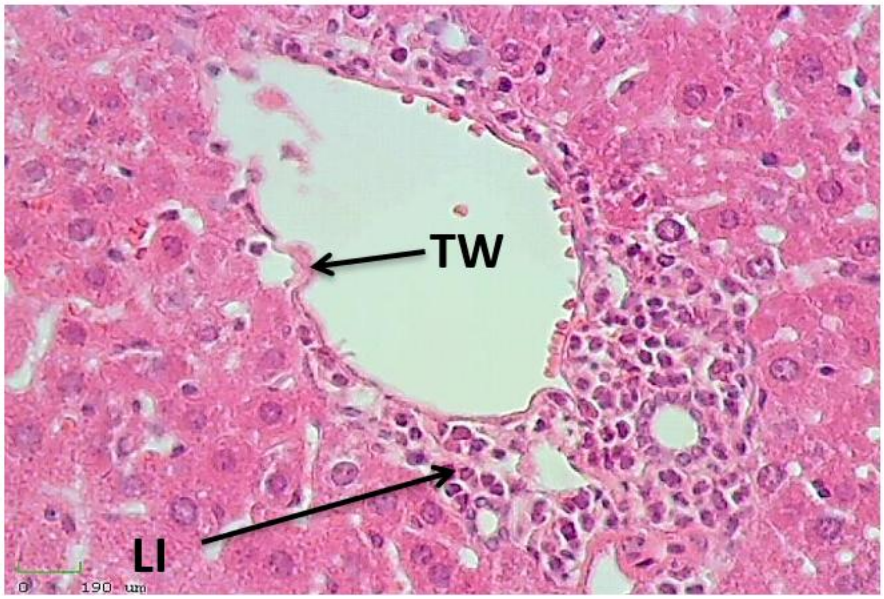

Figure 8: Liver of second group show thickening wall (TW) of central vein and lymphocytes infiltration (LI) H&E X400

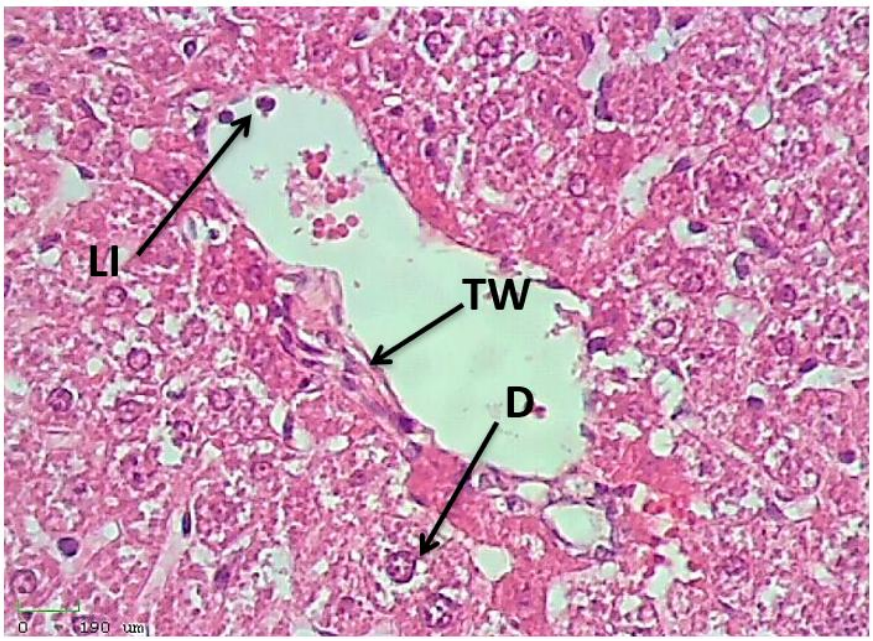

Figure 9: Liver of third group show thickening wall of central vein, degeneration (D) of hepatocytes and lymphocytes infiltration H&E X400

Discussion

Compared to the control group, interleukins showed a significantly higher level of activity. Interleukins are cytokines that are involved in inflammatory processes and stimulate the creation of acute section proteins. These inflammations associated cytokines consist of IL-1α, IL-2, IL-6 (Kushner, 1993) and, possibly, IL-8 (Wigmore, Fearon, Maingay, Lai, & Ross, 1997). Many different kinds of cells may make them, but inflammatory regions are where they're most abundantly found, particularly monocytes and macrophages (Gabay, 2006). Also, the nicely recognized domestically generated markers of irritation are TNF -α and IL-6 (Hamzah & Turki, 2011). The results of existing find out about show an excessive effect of lipase of Aspergillus Niger. on antioxidant. Where, the outcomes are in agreement with Devendran et al, (Devendran & Balasubramanian, 2011) referred Intraperitoneal route of aflatoxin (that extracted from Aspergillus Niger) for 8 days caused massive expand in lipid peroxidation in liver of aflatoxin handled rats, as in contrast to controls, lipid peroxidation is regarded as one of the major key events in mobile damage. Thus, an increase in lipid peroxidation in aflatoxin-treated rats compared to controls may be caused by a complete decrease in the activities of enzymatic and non-enzymatic antioxidants like glutathione peroxidase, superoxide dismutase, and catalase in the liver and kidneys (Millman, London, Sutnick, & Blumberg, 1970). In mice, lipase has been shown to cause a number of histological changes, including megasocieties, necrosis, inflammatory infiltrate, nuclear vacuolation, and cytoplasmic hepatocyte vacuolation (Bourogaa, Jarraya, Damak, & Elfeki, 2015). Along with the observations mentioned above, there is further evidence from recent literature that suggests fungal lipases might trigger oxidative stress and inflammatory reactions in living organisms.

The results of the current experimental are articulate to the fact that, exposure of Aspergillus Niger lipase results in noteworthy biochemical and histological changes in female rats, which reflect its possible involvement in enzyme-induced toxicity. The noted increase in interleukins (IL-1, IL-2, IL-6, and IL-8) also speaks in favor of the stimulation of inflammatory processes, which is supported by recent research that fungal-derived enzymes can be a powerful immunomodulator (Alabdalall et al., 2020; Kulas et al., 2019). These are known well-known cytokines mediators of acute and chronic inflammation which are commonly linked with tissue damage and system-wide immune reactions. The symptoms of elevation of malondialdehyde (MDA) levels, as well as, the altered glutathione (GSH) levels, indicate that there is an elevation of oxidative stress. This goes along with data by Huang et al. (2019) who indicated that fungal lipases are agents of lipid peroxidation by destabilizing lipid cellular membranes. One of the important mechanisms involved in causing cell damage is the oxidative stress, which results in the denaturation of proteins, damage of DNA, and consequently, death of cells. Even more recent findings (El-Korany et al., 2020; Alabdalall et al., 2020) also affirm that fungal metabolites have severe negative effects on antioxidant defense systems and thus enhance tissue damage. The histological changes in the hepatocellular degeneration, thrombosis of vases, and inflammatory cell infiltration are signs of progressive liver destruction. Experimental models that were exposed to fungal toxins and enzymes reported similar pathological features (Durr-

e-Nayab et al., 2018; Kulas et al., 2019). These architectural alterations frequently coincide with the functional degeneration, especially, the liver metabolism and detoxification. Also, the dose change effect as was seen in this experiment supports the significance of the level of exposure in deciding the degree of toxicity. Increased enzyme levels were associated with increased biochemical perturbations and tissue damage corroborating previous results that extended or intense exposure causes permanent tissue damage in cells.

CONCLUSION

Although this study has made significant discoveries, there are various weaknesses that need to be put into consideration during interpretation of the results. The relatively small sample size is one of the main limitations and can diminish the statistical power of the analysis and restrict the applicability of the results. The sample used would have been more robust to do comparisons across the experimental groups and the margin of experimentation would have been minimized by using a larger sample. The redundancy of the subjects can also be increased to improve the reproducibility of the findings and build confidence in the biochemical and histological patterns observed. The other significant weakness is the fact that only female rats were used in the study. Despite the fact that the method was effective in the context of evaluating the sex-specific physiological responses, it limits the comparability of results between sexes. Sexual response to enzymatic stress may be different in males and females because these organisms differ in the regulation of hormones, metabolic activity, and immune responses. Consequently, the results do not have the advantage of a comparative male group, which restricts their generalizability. Another limitation is the time of exposure. The research was carried out over a relatively short time period and they were also interested in acute or subacute effects on Aspergillus Niger lipase. This restricts knowledge on long-range or chronic toxicity whether the biochemical changes and tissue injury seen are reversible or might be attained into irreversible pathological states. Moreover, the examination was mainly conducted on liver and other vital organs including kidneys, lungs, heart, and spleen among others were not thoroughly examined. Because the effects of fungal enzymes can be systemic, the analysis of only one organ is a limited view on the toxicity. The biochemical evaluation was also restricted to the selected markers including interleukins, MDA, and GSH. These are significant parameters, but they are unable to provide a detailed biochemical understanding because of the lack of a larger array of oxidative stress and organ functioning biomarkers, such as superoxide dismutase (SOD), catalase, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Lastly, the absence of molecular studies, including gene expression or signaling pathway studies, limits the knowledge of the specific processes that occur to cause lipase-induced toxicity.

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