https://doi.org/10.48047/AFJBS.6.12.2024.1851-1862

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

TO EXAMINE THE HISTOPATHOLOGICAL CHANGES INDUCED BY *ASPERGILLUS FLAVUS* INFECTION IN THE GASTROINTESTINAL TRACT

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Article History
Volume 6, Issue S12, 2024
Received: 16 May 2024
Accepted : 20 June 2024
Doi:
10.48047/AFJBS.6.S12.2024.1851-1862

ABSTRACT

This study aimed to investigate the effects of *Aspergillus flavus* infection on the gastrointestinal tract (GIT) and the inflammatory response induced in animal models. Different concentrations of Aspergillus flavus were administered to animals, and various parameters such as C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-8 (IL-8), and histopathological changes in the colon were assessed. The results showed that increasing doses of *Aspergillus flavus* led to elevated CRP, IL-6, and IL-8 levels, indicating inflammation in the GIT. However, histopathological examination did not reveal significant changes in the colon, suggesting a potential role of innate immunity in defending against fungal infections. This study provides insights into the inflammatory response in the GIT following *Aspergillus flavus* infection and highlights the importance of further research in this area.

Key words:*A spergillus flavus*, Colon, Histopathological, Innate Immunity.

INTRODUCTION

Gastrointestinal Tract: The digestive system, sometimes referred to as the gastrointestinal tract, is made up of several organs that `cooperate to break down food, absorb nutrients, and expel waste. It encompasses the anus, stomach, small and large intestines, oesophagus, and mouth. Food must be broken down into smaller molecules by the gastrointestinal system in order for the body to absorb them and use them as fuel. It also contributes significantly to the immune system by shielding the body from potentially toxic substances and dangerous microorganisms found in food.

The gastrointestinal tract of humans is a highly regulated, multistage system that foods, whether solid or liquid, are broken down into small molecules via processing, dissolving, and absorption through the epithelial cells and into the bloodstream. Food goes through a number of unit activities after consumption that enable the body to meet its energy needs. The mouth, stomach, small, and large intestines are the four unique reactive portions of the GI tract where these proc- endures are performed [1].

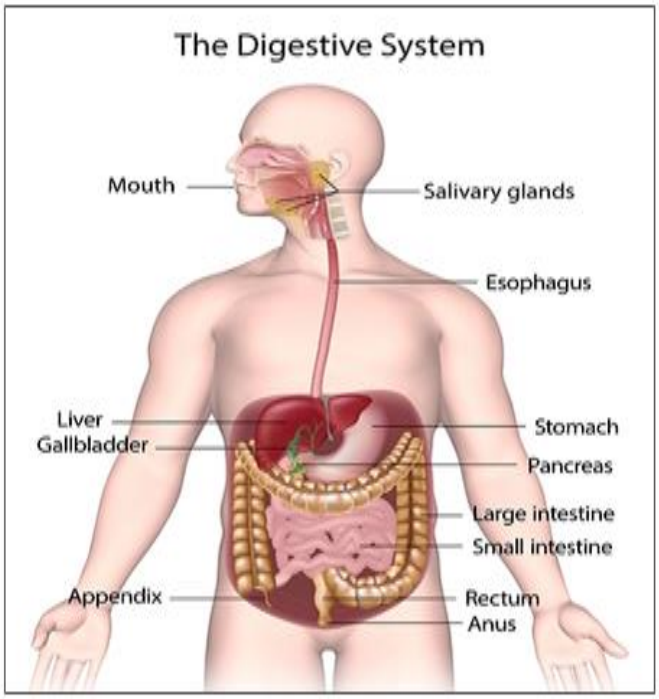

Fig 1. Gastrointestinal Tract[2]

Parts of GIT

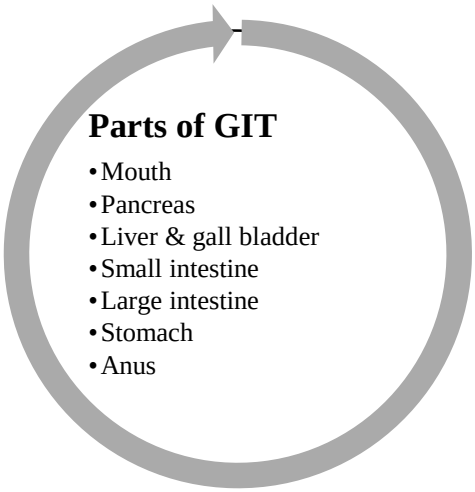

Fig 2. Parts of GIT [3]

Layers of GIT [4]: The gastrointestinal tract wall consists of four highly specialized layers. Each layer has a distinct function and varies along the gastrointestinal tract's length.

- Mucosa
- Submucosa
- Muscular is propria
- Serosa or adventitia

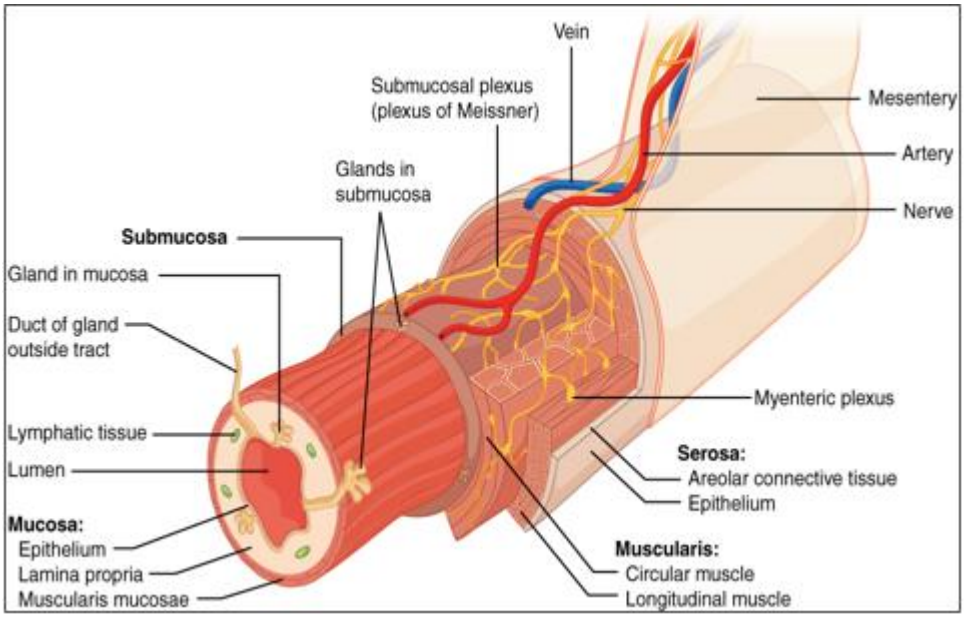

Fig 3. Layers of GIT

***Aspergillus flavus*:** *Aspergillus flavus* is a saprophytic soil fungus that causes aflatoxins, a carcinogenic secondary metabolite, to infect and contaminate preharvest and postharvest seed crops. P. A. Micheli, a Mycologist and Florentine priest, originally defined the genus Aspergillus in 1729. The fungus *Aspergillus flavus* is well-known for its capacity to create aflatoxins, which are strong mycotoxins with proven hepatotoxic and carcinogenic effects. The carcinogenic secondary metabolite aflatoxins is spread by the saprophytic soil fungus *Aspergillus flavus*, which also contaminates preharvest and postharvest seed crops. An established hepatotoxic and carcinogenic fungus species called *Aspergillus flavus* is well-known for its capacity to produce powerful mycotoxins called aflatoxins Aspergillus flavus,a common fungus species found in soil and other substrates, is one of the most significant ones in tropical habitatnumerous human diseases, the most serious of which is invasive aspergillosis, have been linked to *Aspergillus flavus.*

It can also infect insects and causes diseases in crops (peanuts, rice& maize). Poultry feed is mostly made up of agricultural goods, such as a range of oilseeds &cereals including maize, wheat& sorghum, as well as they're by product. When agricultural goods are contaminated with mycotoxin-producing fungi such as Aspergillus Flavus, it can be harmful to both human and animal health. When agricultural goods are contaminated with mycotoxin-producing fungi such as *Aspergillus Flavus*, it can be harmful to both human and animal health. Mycotoxin and toxic fungal contamination cause significant losses in crops and cattle [5, 6].

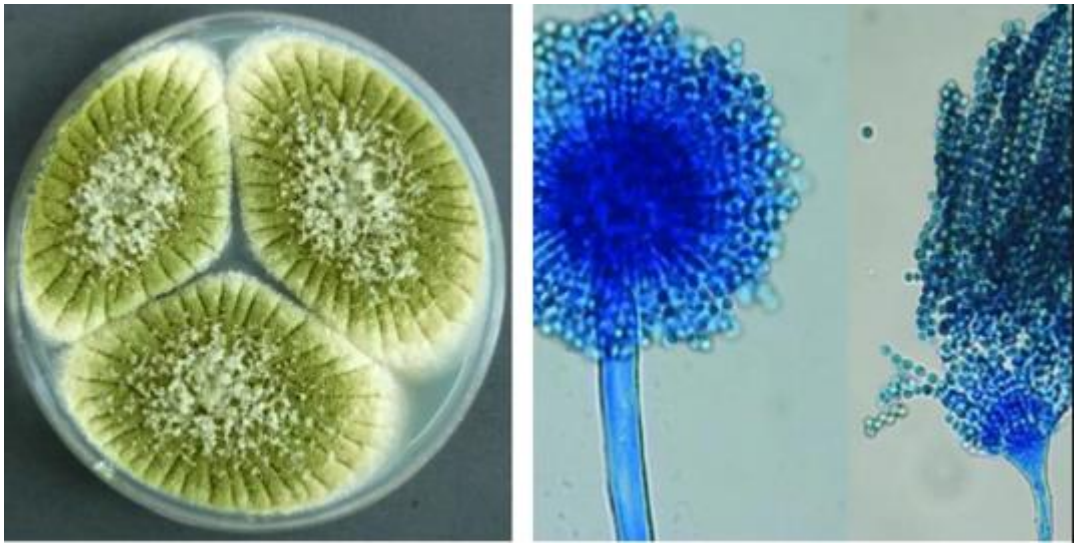

Fig 4. *Aspergillus flavus*

Taxonomical classification of *Aspergillus flavus*

Table 1. Taxonomical classification of *Aspergillus flavus* [7]

Phylum	Ascomycota
Subphylum	Pezizomycotina
Class	Eurotiomycetes
Order	Eurotiales
Family	Trichocomaceae
Genus	*Aspergillus*
Species	*Flavus*

Lifecycle of *Aspergillus flavus*: One of the most common and extensively dispersed soil-borne Molds, *Aspergillus flavus* is present everywhere on the planet. A. flavus is a saprophytic fungus that can live on a variety of organic nutrient sources, including plant debris (tree leaves, decaying wood, animal fodder, cotton, compost piles, dead insects, and animal carcasses), stored grains, indoor and outdoor air environments, and even living people and animals.Two stages comprise the life cycle in agricultural fields: the invasion of seeds and grain in actively developing crop plants [8, 9].

The colonization of plant detritus in the soil. Soil serves as a reservoir for primary inoculum of *A. flavus and A. parasiticus,A. parasiticus* appears to be more adapted to the soil environment, being prominent in peanuts, whereas *A. flavusseems* adapted to the aerial and foliar environment, being dominant in corn, cottonseed, and tree nuts. Under adverse conditions such as dry and poor nutrition, the mycelium congregates to form resistant structures called sclerotia. Sclerotia are pigmented, compacted aggregates of hyphae, which resist unfavourable environmental conditions and are capable of remaining dormant for long periods. The fungus can overwinter as mycelium in plant litter and debris, on insects, or as sclerotia in the soil. Under ideal growing circumstances, the sclerotia either germinate to either more hyphae or conidia, which are asexual spores that can spread through the soil and atmosphere x the fungus primarily takes the form of asexual conidia spores or mycelium [10, 11].

MATERIALS AND EQUIPMENTS [12-14]

Material for Animal Trial

Table 2. Requirement of Animals

Sr. No.	**Species**	**Rat**
1	Strain	Wistar
2	Sex	Male
3	Body weight range	250 – 300 g
4	No. of animals	24

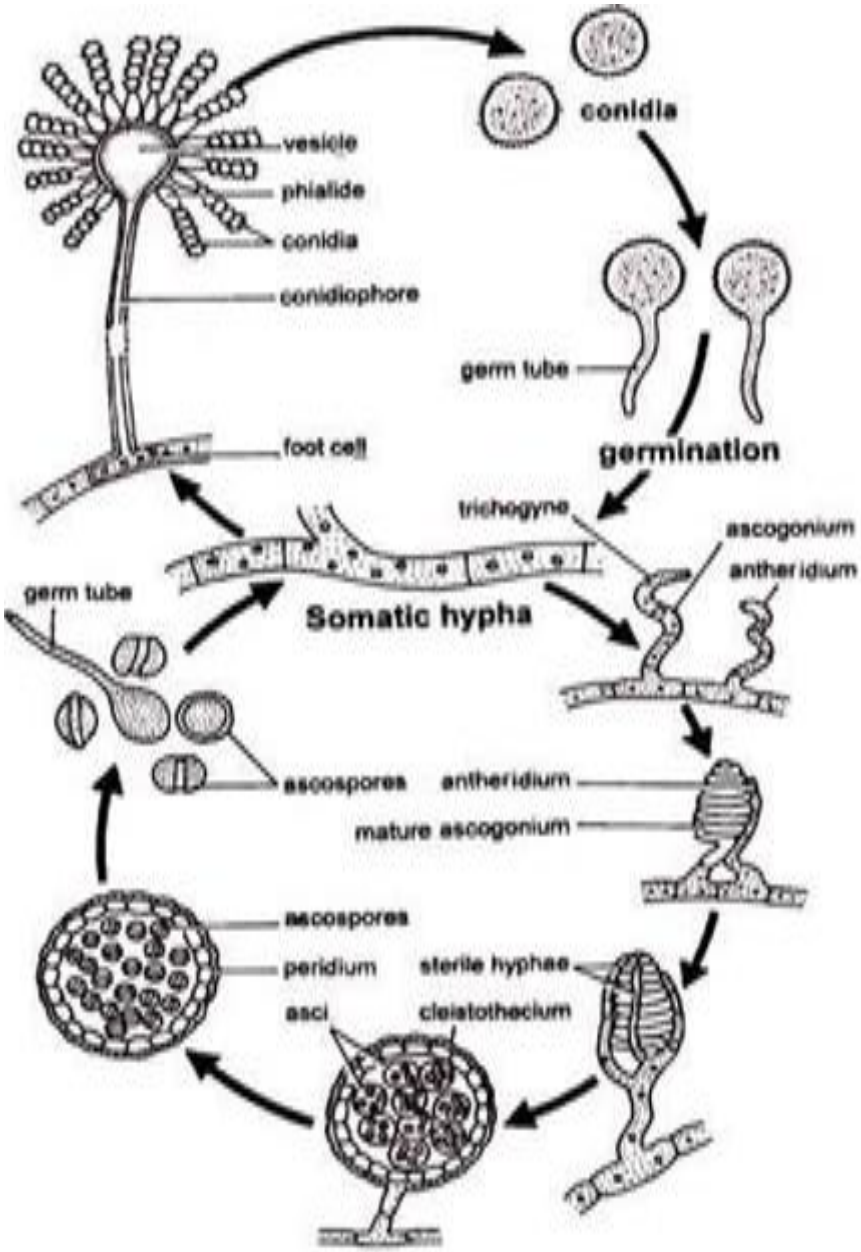

Fig 5. Lifecycle of *Aspergillus flavus*

Animal welfare: 24 Wistar male rats were selected and allowed to acclimatize for period of seven days prior to dosing. During this period, animals were observed daily for clinical signs. All animal met the health and weight criteria

Initial parameter to check:

1. Blood test parameter
   A. CRP Test (C - reactive protein test)
   B. Macrophages and Neutrophil's C.IL 6 and IL8 (ELISA Test).

Final parameter to check

1. Blood Test Parameter:
   A. CRP Test (C - reactive protein test)
   B. Macrophages and Neutrophil's
   C. IL-6 &IL-8 (ELISA Test) 2. Tissue parameter a. Histopathology.
2. Tissue parameter:Histopathology of colon: Ascending Section, Descending section, Transverse Section.

OBSERVATION AND RESULTS

- **Body weight:** Animal body weight changes were observed on weekly basis from acclimatization day to day 10 in the experimental rats.

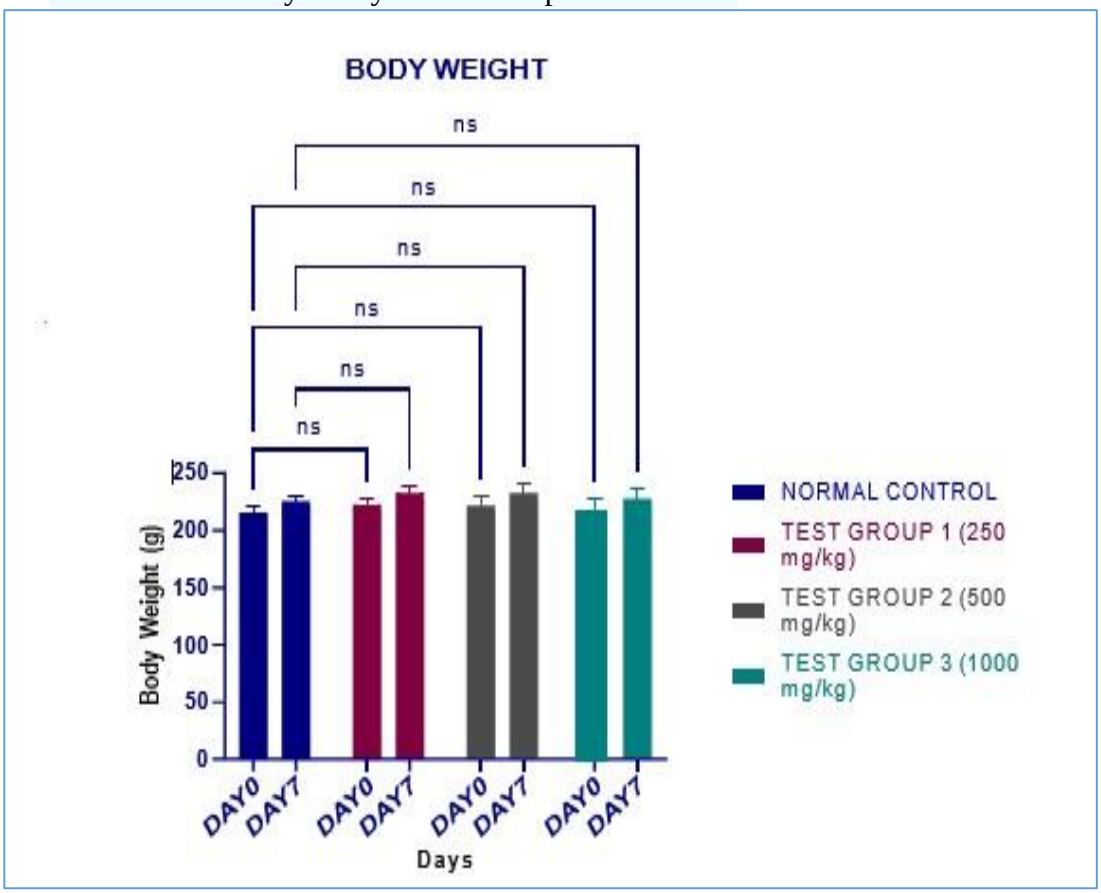

Fig 6. Body Weight

Values are expressed as mean ± SD, n=6, *p<0.05, **p<0.01, *** p<0.001, **** p<0.0001. Values of Groups were compared with Normal Control by t-test. Values of test groups were compared with lowest conc. by Two-way ANOVA by Dunnett's test.

Table 3. Body weight

Groups	**Day 0**	**Day 7**
Normalcontrol	215.667±5.465	225.833±4.708
Testgroup1(250mg/kg)	222.833±5.345	233.333 ±5.680
Testgroup2(500mg/kg)	221.83 ±8.57	233.00 ±7.87
Testgroup3(1000mg/kg)	218.167 ±8.658	228.333 ±7.448

- **Clinical signs and symptoms**

1) CRP

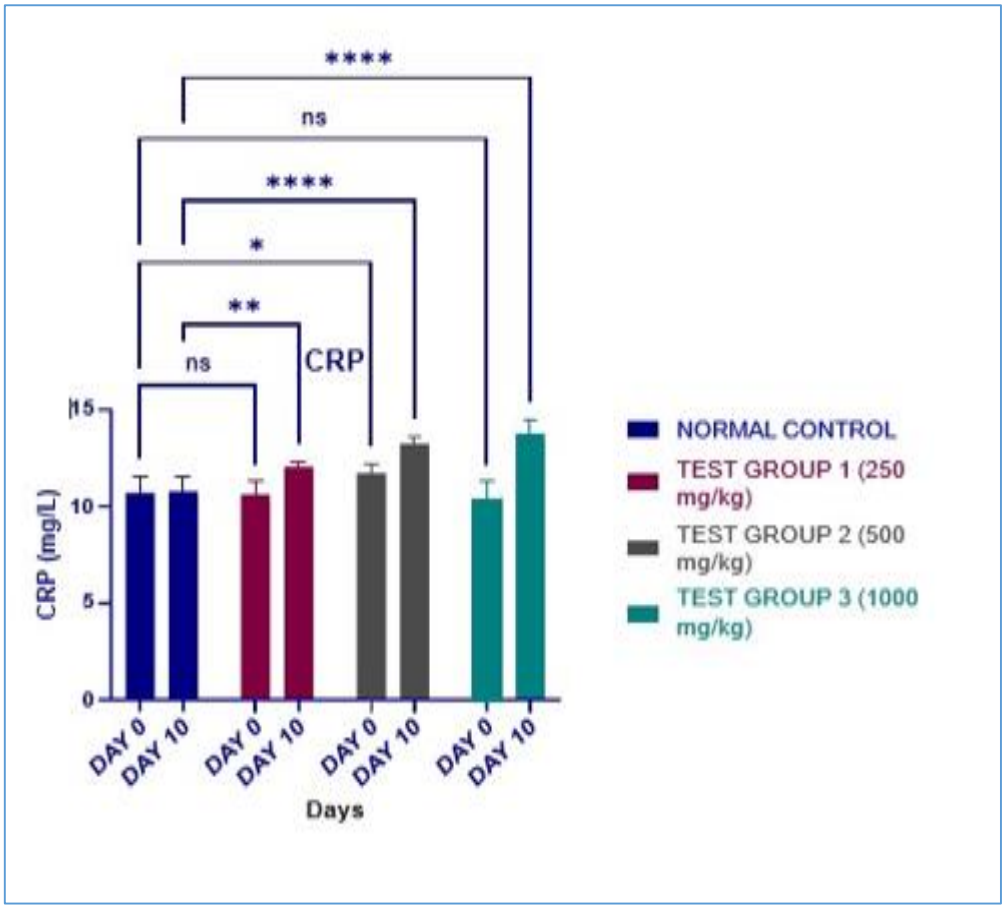

Fig 7. Prism data of CRP

Values are expressed as mean ± SD, n=6, *p<0.05, **p<0.01, *** p<0.001, **** p<0.0001. Groups were compared with Normal Control by t-test. Values of test groups were compared with lowest conc. by Two-way ANOVA by Dunnett's test.

Table 4. CRP

Groups	**Day 0**	**Day10**
Normalcontrol	10.697±0.862	10.785±0.744
Testgroup1(250mg/kg)	10.615±0.662	**12.068 ±0.282
Testgroup2(500mg/kg)	*11.732 ±0.44	****13.268 ±0.36
Testgroup3(1000mg/kg)	10.803±0.492	****13.767 ±0.700

2) IL 6

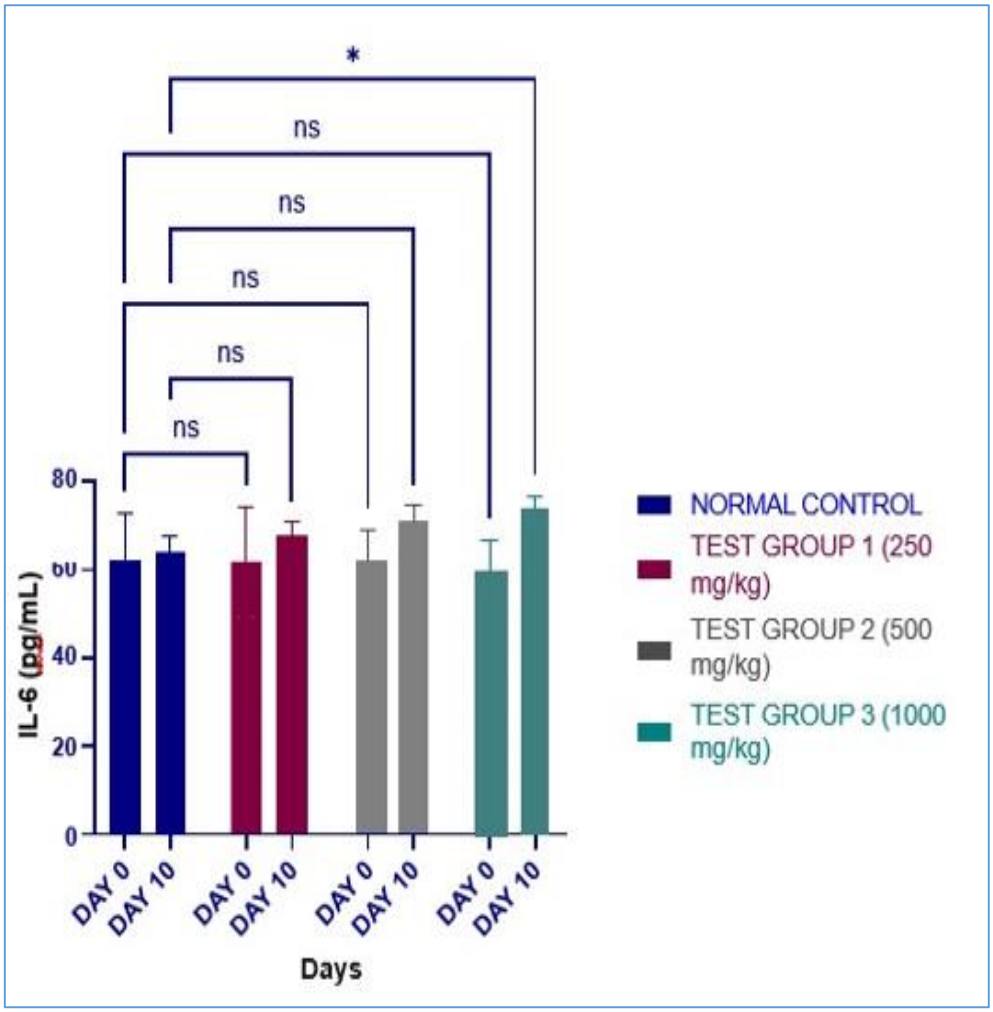

Fig 8. IL 6

Values are expressed as mean ± SD, n=6, *p<0.05, **p<0.01, *** p<0.001, **** p<0.0001. Groups were compared with Normal Control by t-test. Values of test groups were compared with lowest conc. by Two-way ANOVA by Dunnett's test.

Table 5. IL 6

Groups	**Day 0**	**Day10**
Normalcontrol	62.333 ±10.541	64.000 ±3.496
Testgroup1(250mg/kg)	61.778±12.546	67.889±2.722
Testgroup2(500mg/kg)	65.111 ±3.90	72.333 ±2.11
Testgroup3(1000mg/kg)	62.889±3.897	*75.111±1.361

3) IL-8

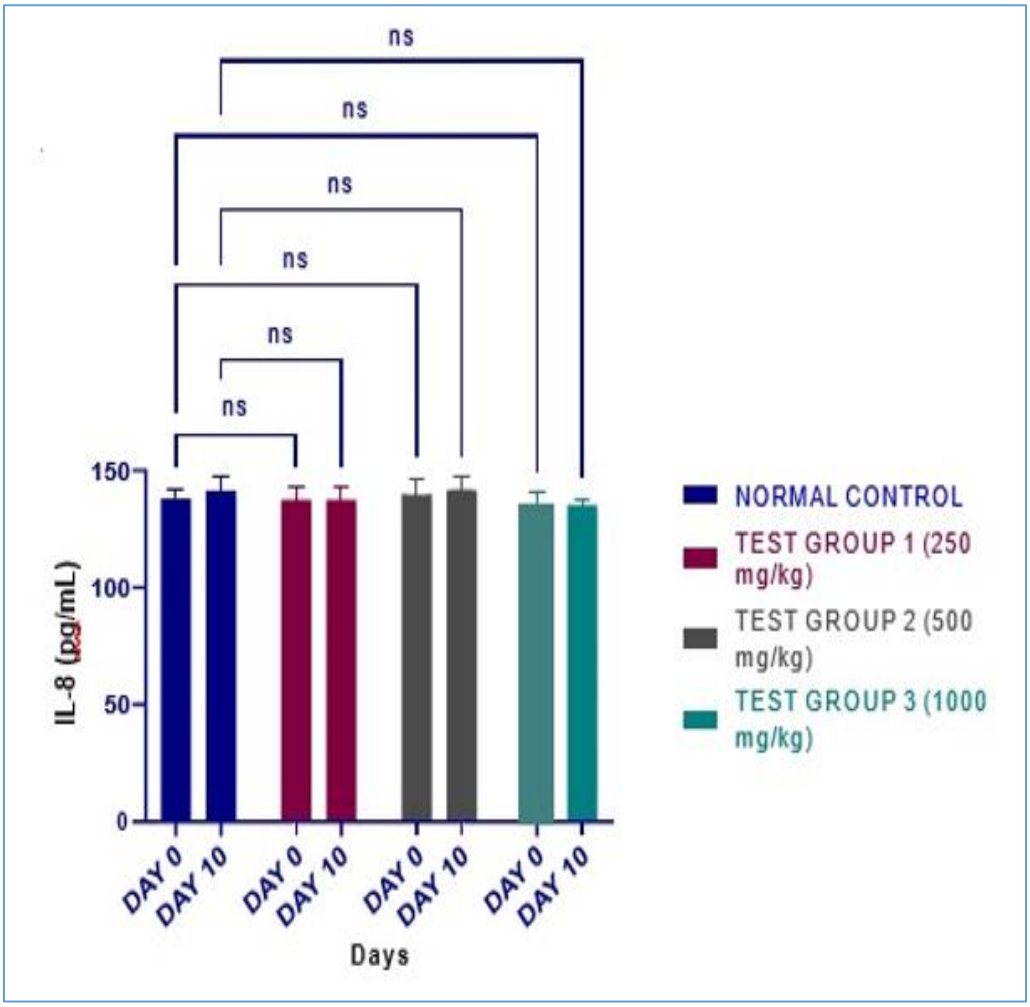

Fig 9. IL-8

Values are expressed as mean ± SD, n=6, *p<0.05, **p<0.01, *** p<0.001, **** p<0.0001. Groups were compared with Normal Control by t-test. Values of test groups were compared with lowest conc. by Two-way ANOVA by Dunnett's test.

Table 6. IL 8

Groups	**Day 0**	**Day10**
Normalcontrol	138.208 ±3.862	141.274±6.351
Testgroup1(250mg/kg)	137.583 ±4.878	137.613±4.910
Testgroup2(500mg/kg)	139.786±6.75	141.810±5.06
Testgroup3(1000mg/kg)	137.375 ±3.103	135.202±2.109

DISCUSSION

Aspergillus flavus is a fungus that can grow on a variety of foods, including grains, nuts, andseeds.When*Aspergillusflavus*growsonfood,itcanproducetoxinscalledaflatoxins.Aflatoxins are harmful to humans and animals and can cause a variety of health problems,includinggastrointestinalproblems.

An animal study can be designed to investigate the effects of *Aspergillus flavus* on thegastrointestinal tract. The study would likely involve dosing animals containing *Aspergillusflavus* and then monitoring the animals for signs of gastrointestinal distress. These signscouldinclude: [15, 16]

- CRPtest(C-reactiveproteintest)
- Macrophages&Neutrophils
- IL-6&IL-8(ELISATest)

The study also involvesexamining the gastrointestinal tract ofthe animals for signs ofdamage,suchasinflammationorbleeding.

- Thedose of*Aspergillusflavus*thatisnecessarytocausegastrointestinalproblems
- Thetypesofgastrointestinalproblemsthatarecausedby*Aspergillusflavus*.
- Themechanismsbywhich*Aspergillusflavus*causegastrointestinalproblems

Theresultsof an animal studyof theeffectsof*Aspergillusflavus*on thegastrointestinaltract could help to improve our understanding of how *Aspergillus flavus* cause disease andcouldlead tothedevelopmentofnew treatments for aflatoxinpoisoning [17, 18].

CONCLUSION

The study concludes that the fungal infection induces an inflammatory response in the gastrointestinal tract (GIT), leading to elevated levels of IL-6 and IL-8.This study concludes that after administering different concentrations of *Aspergillus flavus* to animals, an increase in the dose results in elevated levels of C-reactive protein, IL-6, and IL-8. Therefore, it is concluded that an increase in CRP and interleukin levels indicates inflammation in the GIT.

After observing histopathological changes in the colon, no changes are observed. It can be concluded that after exposure to *Aspergillus flavus,* the body's innate immunity may be playing a crucial role in defending against the fungus.

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