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# Assessing the Dual Roles of Biosulforaphane: Toxicity and Protective Effects against *Aspergillus fumigatus*

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## Abstract

**Background:** *Aspergillus fumigatus* is one of the major pathogenic fungi that produce gliotoxins that acts as the major immuno-suppressive agents which exposes immunocompetent individuals to fatal infection. This study was conducted to look at the toxicity and protective effects of biosulforaphane against invasion of *Aspergillus fumigatus* in immunocompetent albino Wistar rats.

**Methods**: *A. fumigatus* strains were isolated from hospital soil samples and characterized macroscopically, microscopically, and molecularly. Gliotoxin was extracted from yeast extract liquid medium, purified, and detected through Thin Layer Chromatographic (TLC) technique. Biosulforaphane extracted from cabbage was administered orally (0.1-1.0 mL/kg) to Wistar rats for 3 days pre-challenge with fungal suspension ($10^8$ cells/mL). Protective effects on pathology, body/organ weights, and organ functions were assessed using *in vivo* technique."

**Results**: The study revealed that *Aspergillus fumigatus* strain DT0402 (AFDT0), *Aspergillus fumigatus* strain S10 (AFS10) and *Aspergillus fumigatus* strain VIBENF3 (AFVIB) isolated from the soil samples were able to produce gliotoxins. The albino Wistar rats that were administered biosulforaphane had significant ($p \leq 0.05$) protective effects on the clinical manifestations, body weights, organ weights, and organ functions, and 0.5 mL/kg of biosulforaphane proved to be most safe and protective among the studied rats.

**Conclusion**: Therefore, biosulforaphane had significant protective effects against the isolates (AFDT0402, AFS10 and AFVIB) and their gliotoxins of which 0.5 mL/kg of the compound proved to be most safe and effective.

**Keywords**: *Aspergillus,* Gliotoxins, Immunocompetent, Biosulforaphane, Pathological



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## INTRODUCTION

*Aspergillus fumigatus* is one of the significant species naming the genus *Aspergillus*. This species is not just a ubiquitous organism but also a vital pathogenic fungus in clinical setting where the species can cause serious infection called invasive *Aspergillosis*.[1] Aspergillosis is a fungal infection related with high morbidity and mortality rate.[2] *A. fumigatus* has been displayed as the most widely recognized pathogenic species to human host. It is heterotrophic and filamentous, broadly distributed in nature, and generally found in the soil and rotting natural matter. It sporulates bounteously, with each conidial head producing huge number of conidia.[3] At the point when their spores are inhaled, they relocate to the lungs where they sprout, produce nodules in the tissues of the lungs, subsequently disturbing the functions of the respiratory system. The infection becomes invasive whenvarious sections of the different parts of the body are affected, for example, the skin, brain or kidney particularly in people with impaired immune system.[4]

Environments where *Aspergillus fumigatus* can be found include soil, plant materials and refuse dumping sites. This organism has ability of producing airborne spores known as conidia which can be inhaled by individuals in that environment. After inhalation of the spores, they are either killed by immune cells in hosts with energetic immunity, or disease occurs when the immune cells can't eliminate the spores [1]. Aspergillosis is mostly caused by *Aspergillus fumigatus* due to hypersensitive response to the spores, causing harm to the windpipe, and the lungs.[5]

One of the qualities of *A. fumigatus* during early disease development, is Gliotoxin biosynthesis that is triggered in mice.[6] It is hypothesized that the fungus gains from the nutrients supplied by the gliotoxin-annihilated host cells because gliotoxin is cytocidal and immunosuppressive. The gliotoxin's capacity to adapt in the host is demonstrated by its presence in the serum of fungus-infected patients.[3]

Mycotoxin particularly gliotoxin, is one of the most powerful virulent factors, produced by *A. fumigatus*. These virulent factors are proteins that are produced by microorganisms which empowers them to attack the host tissue and cause harm. Virulent factors safeguard them from brutal circumstances which can affect them in the host [6]. Other virulent factors produced by *Aspergillus fumigatus* are adhesins, pigments, hydrolytic enzymes such as proteases, phospholipases, ribonucleases, catalases, superoxide-dismutases, mycotoxins and low-molecular-weight non-protein metabolites.[7] Gliotoxin is a powerful toxin that is produced by pathogenic fungi such as *A. fumigatus*, and shows many immuno suppressive activities, such as blockage of NF-kB activation, inhibition of perforin and Fas ligand dependent killing pathways mediated by cytotoxic T cells, reduction in expression of T Cell receptor (TCR) and CD3, and functions of other immune cells.[8,9] Gliotoxin is an epipolythiodioxopiperazine containing a transannular disulphide bridge. This structure is a vital factor in the metabolite and the organism's harmful effects by the generation of oxygen species that react.[10] Research has indicated that the vast majority of the cruciferous plants, such as; broccoli, cauliflower, brussels sprouts, white and red cabbages and so forth, have the propensity of synthesizing glucoraphanin or glucosinolates which seems to be ineffective in the plants but these plants release isothiocyanates such as biosulforaphane upon hydrolysis of glucoraphanin catalyzed by an enzyme called myrosinase.[11]

In Nigeria, majorly Anambra State, the *in vivo* trial of this natural product (sulforaphane) from cruciferous plants have not been fully studied in order to ascertain the activities of this compound in reducing or preventing the pathogenic potentials of *Aspergillus fumigatus* piloted by gliotoxins secreted by this organism. Although several studies [8, 9, 11, 24] have shown that the compound act on the disulfide bridge of this gliotoxin and metabolize it to an inactivated form but the *in vivo* study of requires proper attention in order to carry out this study. Hence, this study was conducted to look at the toxicity and protective effects of biosulforaphane against invasion of *Aspergillus fumigatus* in immunocompetent albino Wistar rats.

## MATERIALS AND METHODS

***Sample preparation and isolation of the fungal isolates*:** Soil samples collected from hospital dumping sites were used for this study. This was carried out using the modified method of.[12] One gram of the soil sample was weighed into a 50 mL beaker (Pyrex) using analytical weighing balance (JJJ430BC), 3 mL of normal saline (0.85 % NaCl) was added; this was shake thoroughly and made up to 10 mL using the normal saline. Ten-fold serial dilution was done and the sample was aseptically plated on Agar of Sabouraud Dextrosesupplemented with chloramphenicol antibiotics (0.05 %) from the second test tube. This was incubated at room temperature (30± 2 °C) for 5 days.



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***Purification of the isolates:*** Discrete colonies which showed features of *Aspergillus fumigatus* were aseptically subcultured on SDA containing chloramphenicol antibiotics (0.05 %). The subcultured plates were also carefully placed in inverted position, and incubated at room temperature (30 ± 2 °C) for 5 days in order to obtain pure culture.

***Identification of fungal isolates*:** The fungal isolates obtained were identified to the strain level based on molecular characteristics of the isolates obtained from pure cultures.[13]

***Extraction of gliotoxins*:** This was carried out using established method.[14] The test isolates were grown in yeast extract liquid medium containing 20 g of yeast extract, 40 g of sucrose and 1000 mL of distilled water. The biomass generated was macerated in 50mL of chloroform and this was filtered using Whatman No. 1 filter paper. The filtrate was extracted using chloroform and filtered through anhydrous sodium sulphate, $Na_2SO_4$. The chloroform fraction was pooled and evaporated to dryness on a rotary evaporator. The dried extract was then dissolved in the chloroform and stored at 4 °C until analysis.

***Detection of gliotoxins:*** Here, thin layer chromatographic (TLC) technique was used as described by.[14] The plate was prepared using silica gel on microscopic slide (25 mm × 75 mm) and this was allowed to dry at room temperature (30±2 °C) for 24 h. The prepared plate was heated in an oven to activate at 80 °C for 30mins, then 1 cm was drawn from the base and the extracts were carefully dropped at the marked. The plate was developed using toluene/ethylacetate/formic acid (5:4:1/v/v/v). For gliotoxin visualization, developed plate was sprayed with freshly prepared 10 % (w/v) silver nitrate in 80 % (v/v) ethanol. Gliotoxin appeared brown in visible light. The Rf values were also calculated from the distance moved by the analytes and solvents.

***Preparation of test isolate*:** The test isolates were prepared by utilizing the method described by.[15] The isolates were aseptically scraped using normal saline into a cleaned beaker. The suspension of each isolate was centrifuged using an electric centrifuge and filtered. The filtrate from each culture was diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by mixing 0.5 mL of 1.175 % $BaCl_2$. $2H_2O$ and 99.5 mL of 1 % Conc. $H_2SO_4$. The prepared isolates were standardized by comparing the absorbance with that of 0.5 MacFarland standards at 640 nm using UV/visible spectrophotometer.

***Extraction of biosulforaphane*:** This was carried out using the method described by.[16] Twenty-five grams (25 g) of pulverized cabbage floret was weighed using electronic weighing balance (LXB-200C) into a conical flask. This was macerated using 250mL ethanol for 72 h. Then the solution was filtered through filter paper (Whatman N0. 1), and the filtrate was collected in a 250 mL-conical flask (Pyrex). Then 10 mL of dichloromethane (DCM) was added to 25 mL of the extract (filtrate) and mixed thoroughly. This was then centrifuged at 4,000 rpm for 5 mins, and the DCM fraction was decanted and the residue was mixed with 5 mL DCM and mixed thoroughly. This was centrifuged at 4000 rpm for 5 mins, and the DCM fraction was collected and this procedure was repeated three times. The DCM fractions were combined, filtered through cotton wool and dried over anhydrous $Na_2SO_4$. Biosulforaphane was extracted and concentrated using a solid phase extraction column. Biosulforaphane was then eluted with methanol (1.5 mL, 1.0 mL and 0.5 mL). The eluted sample was then exposed for surface evaporation in order to concentrate the compound. The concentrated compound was weighed and resuspended in phosphate buffer saline (PBS) for a concentration of 1.0 mg/mL which was used for the study.

***Experimented animal*:** In this study, the laboratory animal used was albino Wistar rats purchased from the animal house at University of Nigeria, Nsukka (UNN). The rats were transported to the animal house at Department of Biochemistry, Faculty of Biosciences, Nnamdi Azikiwe University (NAU) Awka. The rats were randomly examined for suitability and viability for the study. Those that were not suitable were excluded in the study. The rats were also selected and grouped according to their weights and experimental design.

***Experimental design:*** A total of six (6) albino Wistar rats were randomly selected and exposed to the biosulforaphane (10-fold/5 mL/kg) suspension for 72 h in order to determine the acute toxicity of the compound as described in the study published by.[17] The remaining albino Wistar rats were grouped into four (A, B, C and D). Group A was grouped into four sets, and each set contained four rats. The first set was orally and nasally administered 0.5 mL/kg of AFDT0 suspension whereas the second, third and fourth set were orally administered 0.1 mL/kg, 0.5 mL/kg and 1.0 mL/kg of biosulforaphane solution for 72 h after which the



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suspension (0.5 mL/kg) of AFDT0 was orally and nasally administered to each set respectively. Similar procedure was repeated in group B and C except that AFS10 and AFVIB were administered in group B and C respectively instead of AFDT0. Group D was administered distilled water as normal control. The experimented rats were monitored for three weeks during which the obvious pathological signs were observed and recorded. The body weights of the rats were determined at day 0, 2, 7, 14 and 21 using electronic weighing balance (LXB 200C) as described by.[18] The organs were harvested and examined, their weights were carried out and recorded using electronic weighing balance (LXB 200C) as described in the study published by.[18,19] The kidney function tests were also carried out as described by.[12]

***Statistical Analysis*:** The data generated were expressed in percentages and Tables. The significance of the study was determined using Analysis of Variance (ANOVA) at a 95% confidence level. Post-hoc analysis was carried out, HSD (Honestly Significant Difference) test from IBM SSPS version 30 as described by.[17]

## RESULTS

***Characteristics of the fungal isolates*:** The amplicons obtained after amplification of the extracted DNA displayed cleaned and smooth bands on the agarose gel. The molecular identities of the fungal isolates as shown in Table 1 revealed *Aspergillus fumigatus* strain DT0402 (AFDT0), *Aspergillus fumigatus* strain S10 (AFS10) and *Aspergillus fumigatus* strain VIBENF3 (AFVIB).

**able 1:** Molecular characteristics of the fungal isolates

| Isolate Code | Max score | Total score | Query cover (%) | E value | Identity (%) | Accession Number | Description |
|---|---|---|---|---|---|---|---|
| A | 1306 | 1306 | 100 | 0.0 | 100 | MT316338.1 | *Aspergillus fumigatus* strain DT0402 (AFDT0) small subunit rRNA gene partial sequence |
| B | 1112 | 1112 | 100 | 0.0 | 100 | MH892837.1 | *Aspergillus fumigatus* strain S10 (AFS10) small subunit rRNA gene partial sequence |
| C | 1299 | 1299 | 100 | 0.0 | 100 | MN545449.1 | *Aspergillus fumigatus* strain VIBENF3 (AFVIB) small subunit rRNA gene partial sequence |

***Retention factors of the extracted gliotoxins:*** The retention of the gliotoxins extracted from the strains of *Aspergillus fumigatus* are shown in Table 2. It was observed that the retention factors of the isolates have similar values, although that of AFDT0 was slightly highest followed by that of AFS10 and that of AFVIB was the least. Also, the cleared bands showed after the chromatographic separation showed that the test isolates were able to produce gliotoxins

**Table 2:** Retention factors of gliotoxins extracted from the test isolates

| Isolate | $D_S$ (cm) | $D_A$ (cm) | RF |
|---|---|---|---|
| AFDT0 | 5.60 | 5.40 | 0.96 |
| AFS10 | 5.60 | 5.30 | 0.95 |
| AFVIB | 5.60 | 5.20 | 0.93 |

$D_S$ = Distance moved by the solvent-to-solvent front, $D_A$ = Distance moved by the analyte, RF = Retention Factor, AFDT0 = *A. fumigatus* strain DT0402, AFS10 = *A. fumigatus* strain S10, AFVIB = *A. fumigatus* strain VIBENF3; RFS = Retention factor for reference standard (0.95)

***Lethal toxicity of biosulforaphane:*** The lethal toxicity study revealed that no death was recorded among the animals after 72 h (Table 3). Of 6 albino Wistar rats exposed to the compound, total of six animals survived and no death was recorded.



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**Table 3:** Lethal toxicity of the biosulforaphane in albino Wistar rats

| Time (h) | N | S | D |
|---|---|---|---|
| 24 | 6 | 6 | 0 |
| 48 | 6 | 6 | 0 |
| 72 | 6 | 6 | 0 |

N = Number of animals, S = Number of animals that survived, D = Number of deaths

Effect of biosulforaphane on pathological features of different strains of *Aspergillus fumigatus*

The pathological features were significantly ($\alpha < 0.05$) seen among the infected Wistar rats whereas the protected and normal control rats did not record any significant pathological feature (Table 4, 5 and 6). The infected rats recorded similar number of rats for cough and weight loss but only AFVIB (50 %) showed haemoptysis. One rat each infected with AFDT0 and AFVIB showed runny nose whereas difficulty in breathing was seen most among the rats infected with AFS10. One rat each from those rats infected with AFS10 and AFVIB died before the end of the experiment. The pathological features of the test isolates were most pronounced among the rats infected with AFVIB.

**Table 4:** Pathological manifestation of AFDT0 infected and protected rats

| | | | N=4 | | |
|---|---|---|---|---|---|
| Parameter | AFDT0 | AFDT0 + 0.1 mL/kg | AFDT0 + 0.5 mL/kg | AFDT0 + 1.0 mL/kg | Control |
| Cough | 4 | 0 | 0 | 0 | 0 |
| Haemoptysis | 0 | 0 | 0 | 0 | 0 |
| Weight loss | 4 | 0 | 0 | 0 | 0 |
| Weakness | 4 | 1 | 0 | 1 | 0 |
| Runny nose | 1 | 0 | 0 | 0 | 0 |
| Difficulty in breathing | 1 | 0 | 0 | 0 | 0 |
| Death | 0 | 0 | 0 | 0 | 0 |

AFDT0: *A. fumigatus* strain DT0402; AFDT0S: AFDT0 plus Biosulforaphane; N: Number of rats in each group

**Table 5:** Pathological manifestation of AFS10 infected and protected rats

| p | | | N=4 | | |
|---|---|---|---|---|---|
| Parameter | AFS10 | AFS10 + 0.1 mL/kg | AFS10 + 0.5 mL/kg | AFS10 + 1.0 mL/kg | Control |
| Cough | 4 | 0 | 0 | 0 | 0 |
| Hemoptysis | 0 | 0 | 0 | 0 | 0 |
| Weight loss | 2 | 0 | 0 | 0 | 0 |
| Weakness | 3 | 0 | 0 | 1 | 0 |
| Runny nose | 0 | 0 | 0 | 0 | 0 |
| Difficulty in breathing | 2 | 0 | 0 | 0 | 0 |
| Death | 1 | 0 | 0 | 0 | 0 |

AFS10: *A. fumigatus* strain S10; AFS10S: AFS10 plus Biosulfuraphane;
N: Number of rats in each group.



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**Table 6:** Pathological manifestation of AFVIB infected and protected rats

| | | | N=4 | | |
|---|---|---|---|---|---|
| Parameter | AFVIB | AFVIB + 0.1 mL/kg | AFVIB + 0.5 mL/kg | AFVIB + 1.0 mL/kg | Control |
| Cough | 4 | 0 | 0 | 0 | 0 |
| Haemoptysis | 2 | 0 | 0 | 0 | 0 |
| Weight loss | 4 | 1 | 0 | 1 | 0 |
| Weakness | 3 | 0 | 0 | 0 | 0 |
| Runny nose | 1 | 0 | 0 | 0 | 0 |
| Difficulty in breathing | 1 | 0 | 0 | 0 | 0 |
| Death | 1 | 0 | 0 | 0 | 0 |

AFVIB: *A. fumigatus* strain VIBENF3; AFVIBS: AFVIB plus Biosulforaphane;
N: Number of rats in each group

***Effect of biosulforaphane on body weights of infected rats:*** AFDT0 non-significantly ($\alpha>0.05$) reduced the body weights of the infected rats after 2 days and retarded the increase in weights of the rats, but in the rats protected against AFDT0, their body weights significantly ($\alpha < 0.05$) increased. Similar increase was observed among the control group (Table 7). AFS10 non-significantly ($\alpha>0.05$) retarded the increase in body weights of the infected rats whereas the body weights of those rats protected against the isolates showed significant ($\alpha < 0.05$) increase in body weight as shown in Table 8. AFVIB non significantly ($\alpha>0.05$) reduced the body weights of the infected rats whereas the body weights of the protected rats significantly ($\alpha < 0.05$) increased (Table 9). The study also revealed that administration of 0.5mL/kg among the protected rats showed most pronounced increase in the body weights and this was non-significant ($\alpha>0.05$) when compared to the effects of 0.1mL/kg and 1.0 mL/kg. The study also showed that administration of 0.5 mL/kg restored the body weights state of the experimented rats, and this was followed by 1.0 mL/kg as compared to the normal control group.

**Table 7:** The effect of AFDT0 in the body weights of the protected and unprotected rats

| **Day** | **Control** | **AFDT0** | **AFDT0 plus 0.1 mL/kg** | **AFDT0 plus 0.5 mL/kg** | **AFDT0 plus 1.0 mL/kg** |
|---|---|---|---|---|---|
| 0 | 122.01±1.31 | 122.06±1.29 | 121.96±1.44 | 122.08±1.58 | 122.19±1.17 |
| 2 | 128.42±0.69 | 119.57±1.98 | 126.61±1.51 | 128.35±0.73 | 125.97±1.38 |
| 7 | 135.69±1.22 | 122.36±1.79 | 130.39±1.10 | 135.22±1.40 | 133.13±2.60 |
| 14 | 143.80±1.22 | 128.78±1.79 | 143.55±1.10 | 143.55±1.40 | 141.35±2.60 |
| 21 | 155.80±1.17 | 127.15±0.67 | 149.20±1.07 | 152.90±2.22 | 152.71±2.02 |

AFDT0 = *A. fumigatus* strain DT0402

**Table 8:** The effect of AFS10 in the body weights of protected and unprotected rats

| **Day** | **Control** | **AFS10** | **AFS10 plus 0.1 mL/kg** | **AFS10 plus 0.5 mL/kg** | **AFS10 plus 1.0 mL/kg** |
|---|---|---|---|---|---|
| 0 | 122.01±1.31 | 121.78±1.26 | 121.87±1.40 | 121.88±1.28 | 121.85±1.26 |
| 2 | 128.42±0.69 | 124.33±2.64 | 125.77±0.99 | 127.29±0.79 | 126.44±1.02 |
| 7 | 135.69±1.22 | 126.12±4.27 | 131.24±1.14 | 133.96±1.10 | 131.65±1.26 |
| 14 | 143.80±1.75 | 127.67±1.17 | 140.22±0.67 | 141.49±1.33 | 140.45±1.01 |
| 21 | 155.80±1.17 | 124.80±1.17 | 149.39±0.67 | 152.88±2.02 | 151.55±1.37 |



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AFS10 = *A. fumigatus* strain S10

**Table 9:** The effect of AFVIB on the body weights of protected and unprotected rats

| Day | Control | AFVIB | AFVIB plus 0.1 mL/kg | AFVIB plus 0.5 mL/kg | AFVIB plus 1.0 mL/kg |
|---|---|---|---|---|---|
| 0 | 122.01±1.31 | 121.93±1.12 | 121.93±1.41 | 122.25±1.47 | 122.03±1.29 |
| 2 | 128.42±0.69 | 120.26±2.26 | 124.70±0.97 | 126.72±0.96 | 125.88±0.66 |
| 7 | 135.69±1.22 | 115.23±2.18 | 128.44±1.01 | 132.25±0.94 | 129.93±1.37 |
| 14 | 143.80±1.75 | 119.07±2.00 | 136.66±1.32 | 139.82±0.81 | 139.09±2.23 |
| 21 | 155.80±1.17 | 122.15±1.20 | 146.51±2.24 | 152.44±0.46 | 148.01±1.03 |

AFVIB = *A. fumigatus* strain VIBENF3

***Effect of biosulforaphane on organ function of infected rats:*** The lungs and kidney of the infected groups were mostly reduced compared to the lungs and kidney from protected and normal control group (Table 10). The lungs of the group infected with AFVIB were mostly reduced, and the kidneys of the group infected with AFS10 were mostly reduced. It was also observed that the group protected with 0.5 mL/kg of biosulforaphane showed most increased in the weights of lungs and kidney whereas those administered 0.1 mL/kg showed the least increase among the protected groups. Statistically, there was no significant difference ($\alpha>0.05$) between the control normal group and the protected and infected groups but deviations were seen in their weights. The urea and creatinine levels from the kidney of infected groups were non-significantly ($\alpha>0.05$) higher than that of protected and normal control groups (Table 11). The urea and creatinine level were mostly secreted from the group infected with AFS10, and this was significantly ($\alpha<0.05$) reduced when the rats were protected with biosulforaphane, of which 0.5 mL/kg proved to be most effective. Similar trends were observed among other groups infected with AFDT0 and AFVIB, and those protected with biosulforaphane against these organisms.

**Table 10:** Effects of the *Aspergillus fumigatus* strains on the weights of lungs and kidney of infected and protected rats

| Design | Lungs (g) | Kidney (g) |
|---|---|---|
| AFDT0 | 0.73±0.00 | 0.41±0.00 |
| AFS10 | 0.68±0.00 | 0.36±0.00 |
| AFVIB | 0.66±0.00 | 0.38±0.00 |
| AFDT0+0.1 | 0.88±0.00 | 0.48±0.00 |
| AFDT0+0.5 | 1.02±0.00 | 0.59±0.00 |
| AFDT0+1.0 | 0.97±0.00 | 0.56±0.00 |
| AFS10+0.1 | 0.79±0.00 | 0.42±0.00 |
| AFS10+0.5 | 0.93±0.00 | 0.49±0.00 |
| AFS10+1.0 | 0.85±0.00 | 0.44±0.00 |
| AFVIB+0.1 | 0.84±0.00 | 0.46±0.00 |
| AFVIB+0.5 | 0.98±0.00 | 0.51±0.00 |
| AFVIB+1.0 | 0.92±0.00 | 0.47±0.00 |
| Control | 1.10±0.00 | 0.61±0.00 |

AFDT0 = *A. fumigatus* strain DT0402, AFS10 = *A. fumigatus* strain S10, AFVIB = *A. fumigatus* strain VIBENF3

**Table 11:** Effects of *Aspergillus fumigatus* strains on kidney function of the studied rats

| Design | Urea (mg/dL) | Creatinine (mg/dL) |
|---|---|---|
| AFDT0 | 11.0847 | 0.4884 |
| AFS10 | 18.1456 | 0.5210 |
| AFVIB | 14.2468 | 0.4898 |
| AFDT0+0.1 | 9.8820 | 0.4780 |
| AFDT0+0.5 | 8.5640 | 0.4446 |
| AFDT0+1.0 | 9.0472 | 0.4682 |



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| Design | Urea (mg/dL) | Creatinine (mg/dL) |
|---|---|---|
| AFS10+0.1 | 10.8224 | 0.4712 |
| AFS10+0.5 | 10.0263 | 0.4701 |
| AFS10+1.0 | 10.3112 | 0.4708 |
| AFVIB+0.1 | 10.0108 | 0.4690 |
| AFVIB+0.5 | 9.2001 | 0.4041 |
| AFVIB+1.0 | 9.7202 | 0.4248 |
| Control | 7.9812 | 0.3926 |

AFDT0 = *A. fumigatus* strain DT0402, AFS10 = *A. fumigatus* strain S10, AFVIB = *A. fumigatus* strain VIBENF3

## DISCUSSION

The present study has shown that the retention factors gliotoxins extracted from *A fumigatus* had similar values with the value of the reference standard. This shows that the fumigatus isolates (AFDT0, AFS10, AFVIB) excreted reasonable amount of gliotoxins. The extraction and detection of gliotoxins among the *Aspergillus fumigatus* isolates agrees with findings of.[9,14,20] Several researchers have pointed that *Aspergillus fumigatus* produced gliotoxins as their major virulence factors, and variability of environmental factors such as moisture, temperature, water activity, nutrient or good oxygenation may lead to activation of genes responsible for production of gliotoxin.[21,22,23]

The pathological features (cough, haemoptysis, weight loss, weakness, runny nose) exhibited by the infected rats were significantly reduced/restored in those rats protected with biosulforaphane. [24] stated that Bak-proteins induced apoptotic activity of gliotoxins and this cause deformation and deaths of lungs and kidney cells. Significant increase in the weights of the lungs and kidney, and reduction in urea and creatinine level observed among the rats administered biosulforaphane could be attributed to the fact that biosulforphane protect cells and organs against oxidative stress and deformation from pathogenic organisms. Similar conclusion was drawn by.[25] Also,[26] pointed that biosulforphane (i-isothiocyanate-4-methylsulfonylbutane) is a plant extract obtained from cruciferous vegetables that exert antioxidant and anti-inflammatory effects. This pointed the ability of the sulforphane to decrease nuclear factor-kB (NF-kB) expression in *Aspergillus fumigatus* and this inhibited the secretion of gliotoxin in the protected rats. Similar conclusion was drawn by.[26] Also, the protective activity of the biosulforaphane could be attributed to its ability to stimulate endogenous detoxifying enzymes such as glutathione-S-transferase and quinine reductase which destroyed the test isolates and the by-products (gliotoxins) in the protected rats. Similar finding was reported by.[27]

The study demonstrates the protective effects of biosulforaphane against *Aspergillus fumigatus*-induced toxicity in immunocompetent albino Wistar rats, highlighting its potential as a therapeutic agent against fungal infections. A key strength of the study is the use of a relevant animal model and multiple strains of *A. fumigatus,* increasing the generalizability of the findings. However, a limitation is the lack of mechanistic insights into biosulforaphane's protective effects, and the use of a single administration route (oral) and duration (3 days) may not fully capture its therapeutic potential. Additionally, the study's focus on gliotoxin-producing *A. fumigatus* strains may not represent the full spectrum of *Aspergillus* pathogenesis.

The study's findings imply that biosulforaphane, a cabbage-derived compound, may offer a promising therapeutic strategy against *Aspergillus fumigatus* infections, particularly in immunocompetent individuals. The significant protective effects against gliotoxin-induced toxicity, improved clinical outcomes, and organ function suggest potential applications in prevention and treatment of fungal infections, possibly through dietary interventions or supplements. The identification of a safe and effective dose (0.5 mL/kg) warrants further investigation into biosulforaphane's mechanisms and efficacy in humans, potentially informing novel approaches to mitigate the impact of *A. fumigatus* infections, especially in high-risk environments like hospitals.

## CONCLUSION

The study has revealed that isolates; *Aspergillus fumigatus* strain DT0402 (AFDT0), *Aspergillus fumigatus* strain S10 (AFS10) and *Aspergillus fumigatus* strain VIBENF3 (AFVIB) were able to produce gliotoxins. The albino Wistar rats that were administered biosulforaphane had



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significant protective effects on the clinical manifestations, body weights, organ weights, and organ functions, and 0.5 mL/kg of biosulforaphane proved to be safer and more protective among the studied rats.

**Declarations**

***Conflicting Interest Statement:*** We declare that we have no conflict of interest

***Ethical Approval*:** All authors hereby declare that "Principles of laboratory animal care" (NCARE with Ref No FPSRA/UNN/24/0111), certified on 5th November, 2024 at University of Nigeria, Nsukka, were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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