



## Hydroxychloroquine and zinc ameliorate interleukin-6 associated hepato-renal toxicity induced by *Aspergillus fumigatus* in experimental rat models

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### Abstract:

**Background:** In Nigeria, immunocompromised persons, particularly those living with HIV, are at an increased risk of developing invasive pulmonary aspergillosis caused by *Aspergillus fumigatus*. Interestingly, this condition produces symptoms that can be easily mistaken for those of COVID-19. This misdiagnosis results in their treatment with zinc and hydroxychloroquine (HCQ). To better understand the pathophysiology of aspergillosis and determine the therapeutic and toxic effects of zinc and HCQ, this study examined liver and renal functions in experimental rat models.

**Methodology:** Twenty-eight Albino rats, randomised into 7 groups (n=4 each) designated A to G, were used for this study. Group A rats received standardized rat chow and distilled water only. Group B rats received moderate dose of HCQ only. Group C to G rats received immunosuppressive agents (an alkylating agent: cyclophosphamide and a steroid: hydrocortisone) to simulate an immunocompromised state before being infected with *A. fumigatus* suspension (AFS). Group C rats received AFS without treatment. Group D rats simultaneously received AFS and low dose of HCQ. Group E rats simultaneously received AFS and moderate dose of HCQ. Group F rats simultaneously received AFS and high dose of HCQ, and Group G rats simultaneously received AFS and moderate dose of HCQ and zinc. Serum levels of interleukin (IL)-6 and IL-10, liver enzymes, and renal parameters were measured using standard methods. The weights of the lungs, liver, and kidneys of each rat were measured after being sacrificed. One-way analysis of variance (ANOVA) was used to compare the means ( $\pm$ SD) of the biochemical variables and relative weight of the organs, while Post Hoc test was used for group comparison. Pearson's correlation was used to determine relationship between parameters, with significant levels established at  $p < 0.05$ .

**Results:** Higher levels of serum alanine transaminase, creatinine, and urea and lower relative lung weight were observed in group C rats (infected but untreated) compared to rats in other groups ( $p < 0.001$ ). Higher IL-6 levels and IL-6/IL-10 ratio were also observed in group C rats compared to rats in other groups ( $p > 0.05$ ).

**Conclusion:** This study revealed that HCQ and zinc ameliorate oxidative stress and hepato-renal damage induced by *A. fumigatus* in Albino rats.

**Keywords:** Cytokine; Oxidative stress; Aspergillosis; Interleukin-6; Interleukin-10

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## L'hydroxychloroquine et le zinc améliorent la toxicité hépato-rénale associée à l'interleukine-6 induite par *Aspergillus fumigatus* dans des modèles expérimentaux de rats

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### Résumé:

**Contexte:** Au Nigeria, les personnes immunodéprimées, en particulier celles vivant avec le VIH, courent un risque accru de développer une aspergillose pulmonaire invasive causée par *Aspergillus fumigatus*. Il est intéressant de noter que cette maladie produit des symptômes qui peuvent facilement être confondus avec ceux du COVID-19. Cette erreur de diagnostic entraîne leur traitement au zinc et à l'hydroxychloroquine (HCQ). Pour mieux comprendre la physiopathologie de l'aspergillose et déterminer les effets thérapeutiques et toxiques du zinc et de l'HCQ, cette étude a examiné les fonctions hépatiques et rénales dans des modèles expérimentaux

de rats.

**Méthodologie:** Vingt-huit rats albinos, randomisés en 7 groupes (n=4 chacun) désignés de A à G, ont été utilisés pour cette étude. Les rats du groupe A ont reçu uniquement de la nourriture pour rats standardisée et de l'eau distillée. Les rats du groupe B ont reçu uniquement une dose modérée de HCQ. Les rats des groupes C à G ont reçu des agents immunosuppresseurs (un agent alkylant: cyclophosphamide et un stéroïde: hydrocortisone) pour simuler un état d'immunodépression avant d'être infectés par une suspension d'*A. fumigatus* (AFS). Les rats du groupe C ont reçu de l'AFS sans traitement. Les rats du groupe D ont reçu simultanément de l'AFS et une faible dose de HCQ. Les rats du groupe E ont reçu simultanément de l'AFS et une dose modérée de HCQ. Les rats du groupe F ont reçu simultanément de l'AFS et une dose élevée de HCQ, et les rats du groupe G ont reçu simultanément de l'AFS et une dose modérée de HCQ et de zinc. Les taux sériques d'interleukine (IL)-6 et d'IL-10, les enzymes hépatiques et les paramètres rénaux ont été mesurés à l'aide de méthodes standard. Le poids des poumons, du foie et des reins de chaque rat a été mesuré après sacrifice. Une analyse de variance unidirectionnelle (ANOVA) a été utilisée pour comparer les moyennes ( $\pm$ SD) des variables biochimiques et du poids relatif des organes, tandis que le test Post Hoc a été utilisé pour la comparaison de groupes. La corrélation de Pearson a été utilisée pour déterminer la relation entre les paramètres, avec des niveaux significatifs établis à  $p < 0,05$ .

**Résultats:** Des taux sériques plus élevés d'alanine transaminase, de créatinine et d'urée et un poids relatif inférieur des poumons ont été observés chez les rats du groupe C (infectés mais non traités) par rapport aux rats des autres groupes ( $p < 0,001$ ). Des taux d'IL-6 et un rapport IL-6/IL-10 plus élevés ont également été observés chez les rats du groupe C par rapport aux rats des autres groupes ( $p > 0,05$ ).

**Conclusion:** Cette étude a révélé que l'HCQ et le zinc atténuent le stress oxydatif et les lésions hépato-rénales induites par *A. fumigatus* chez les rats albinos.

**Mots clés:** Cytokine; Stress oxydatif; Aspergillose; Interleukine-6; Interleukine-10

## Introduction:

*Aspergillus* is a filamentous mold that may cause a wide spectrum of infections including acute life-threatening infections, chronic pulmonary infections, hypersensitivity and allergic diseases depending on the host immune status or pulmonary structure (1). Aspergillosis is caused by the species of mold called *Aspergillus fumigatus*. An invasive variant of aspergillosis is a devastating illness, with mortality rates in some patient groups reaching as high as 90% (2). Over the previous two decades, the incidence of invasive aspergillosis has increased tenfold (3). Aspergillosis is seen in immunocompromised patients and can also be symptomatic in certain cases. A prevalence of 3.1% was reported among individuals living with HIV in Nigeria (4).

Studies have shown that hydroxychloroquine (HCQ) modulates the immune system by interfering with lysosomal acidification, inhibition of antigen presentation, and down-regulation of cytokine production and secretion by monocytes and T-cells (5). Hydroxychloroquine inhibits cytokine production and modulation of certain co-stimulatory molecules (6) while zinc is an essential vital exogenous mineral that aids DNA synthesis, enzymatic reactions, immune functions, protein synthesis, wound healing, growth, and development (7).

Inflammatory cytokines such as interleukin (IL)-6 and IL-10 are important biomarkers for distinguishing infections caused by various pathogens (8). IL-6 and interferon gamma (IFN- $\gamma$ ) were reported to be predominantly elevated in invasive pulmonary aspergillosis and *Pneumocystis pneumonia* (8). IL-10 increases the host susceptibility to lethal fungal infection, possibly because IL-10 is asso-

ciated with  $T_{H2}$  response, down-regulation of  $T_{H1}$  response, and macrophage activation (9).

With the rising frequency of pulmonary aspergillosis diagnosis and chronic obstructive pulmonary disease in Nigeria, it has become critical to assess the efficacy of HCQ and zinc in alleviating respiratory distress associated with pulmonary aspergillosis. For the first time, this study investigated the curative and toxic effects of different doses of HCQ and zinc on experimentally induced pulmonary aspergillosis and respiratory distress in Albino rats following exposure to *A. fumigatus*.

## Materials and method:

### Study site and ethical considerations:

This experimental study was carried out at the vivarium in the Department of Physiology, Nnamdi Azikiwe University, Nnewi Campus, Nigeria. Ethical approval for the research was obtained from the College of Health Sciences Ethics Committee at the Nnamdi Azikiwe University (NAU/FHST/2021/MLS68 and NAU/FHST/2021/MLS105). The rats were carefully handled in line with the 2011 ethical standards and protocols for the care and use of laboratory animals of the National Institute of Health.

### Collection of *Aspergillus* isolate for induction of aspergillosis:

*Aspergillus fumigatus* isolate used in the study was obtained from a patient sample in Onitsha and this was sub-cultured on Sabouraud dextrose agar (SDA) at 37°C for 2 days. The culture growth was identified microscopically following the Lactophenol cotton blue (LCB) staining technique as described by Moore and Jaciow (10). The conidia were extracted by washing the plates with sterile

0.2% Tween 20 and Normal Saline, followed by centrifugation and filtration of the suspension.

#### Median effective dose (ED50) of the drugs used in the study:

The oral LD<sub>50</sub> of HCQ in rats is reported to be 1240 mg/kg while the therapeutic dose is 10 mg/kg (11). The oral LD<sub>50</sub> of zinc salts in rats is 237–623 mg/kg (12) while the LD<sub>50</sub> of hydrocortisone acetate is 150 mg/kg. The intraperitoneal LD<sub>50</sub> of cyclophosphamide mixed with halothane is 152 mg/kg (13). Both cyclophosphamides mixed with halothane and hydrocortisone were used as immunosuppressive agents.

#### Study design and animal handling:

The test system for this study was Albino rats (n=28) weighing 80±20g (Table 1). The rats were allowed to acclimatize in the vivarium for two weeks before the start of the experiment and their daily consumption of food was noted. They were then randomized into seven groups (A-G), with each group containing four rats.

On day 1, rats in groups C to G were given moderate dose of steroid-sparing agent (cyclophosphamide 75 mg/kg, ~0.3 ml intraperitoneally) and steroid (hydrocortisone acetate 80 mg/kg, ~0.32 ml intraperitoneally) to simulate immunocompromised state, replicating real-life situation as aspergillosis, which majorly affects immunosuppressed individuals, thus facilitating the development of the disease. The steroids were administered with syringes while HCQ and zinc were administered orally with an oral cannula.

On day 6, the rats were anaesthetized with 0.5 ml ketamine, administered slow-

ly over 60 seconds. Still on day 6, aspergillosis was sufficiently induced with *Aspergillus* suspension in drops into the right nasal cavity using a tuberculin syringe, with the rat nostrils pointed facing upwards. The suspension of *A. fumigatus* conidia was dropped at a slow rate (150 µl) into the right nasal cavity with the tuberculin string gently inserted 1.5 cm deep into the nasal cavity.

On day 7, a second batch of immunosuppressants was administered (cyclophosphamide intraperitoneally at a dose of 60 mg/kg) to ensure immunosuppression. On days 8, 9, and 10, rats were also exposed to *A. fumigatus* again via inhalation of the conidia spore. Starting from day 13 to day 15, the respective rats (groups D-G) were given their corresponding doses of HCQ and zinc. On day 16, the rats were made to fast and then sacrificed on day 17.

#### Sample collection and laboratory analysis:

On day 17, the rats were anaesthetized by placing them in an air-tight jar containing cotton wool soaked in chloroform. Following loss of reflexes, response to stimulus, and reduction in the animal respiratory rate, blood samples were collected by ocular puncture. The blood samples were collected into EDTA (Alpha Surgicare) and plain bottles, labelled and left undisturbed for 15 minutes. The whole blood samples were centrifuged for 10 minutes at 3000 rpm to separate the serum.

The lungs, kidneys, and liver of the rats were subsequently harvested, processed, and stained using the Haematoxylin and Eosin (H & E) staining technique as described by Feldman and Wolfe (14).

Table 1: Description and regimen for rats in the control and experimental groups

Groups of rats	Description	Treatment
Control	Group A	Neutral control
	Group B	Negative control
	Group C	Positive control
Experimental	Group D	Treatment
	Group E	Treatment
	Group F	Treatment
	Group G	Treatment

All animals received standard feed and distilled water *ad libitum*

### Determination of serum levels of interleukins 6 and 10:

Before the biochemical assays were carried out, all reagents, serum, calibrators, and controls were brought to room temperature (27°C). The serum levels of IL-6 and IL-10 were determined using ELISA Micro-well Test Kit at 450 nm absorbance in a micro-plate reader. The IL-6 ELISA kit Fine (Test ER0042) possessed a reactivity range of 62.5 - 4000 pg/ml and a sensitivity of 37.5 pg/ml, while the IL-10 ELISA test kit Fine (Test ER0033) possessed a reactivity of 31.25 - 2000 pg/ml and a sensitivity of 18.75 pg/ml.

### Statistical analysis:

The Statistical Package for the Social Sciences (SPSS) version 25.0 and GraphPad Prism (version 6.0) were used to analyze the data. One-way analysis of variance (ANOVA) was used to compare the means ( $\pm$ SD) of the variables from the biochemical assay as well as relative organ weights (organ weight over total body weight multiplied by 100).

The Post Hoc test was used for group comparison. To determine the relationships between parameters, Pearson's correlation was used. Data were considered significant at  $p \leq 0.05$ ,  $\leq 0.01$ , and  $\leq 0.001$ . Relative organ weight was calculated as the weight of the organ (lungs, liver, and kidney) divided by total body weight multiplied by 100.

### Results:

Following intraperitoneal injection of cyclophosphamide and hydrocortisone acetate, experimental rats in groups C, D, E, F, and G had decreased appetite; a reduction in consumption of daily ration compared to the amount consumed during the acclimatization period. Four days post-induction of immunosuppression, the rats exhibited lethargy and decrease in their reflexes. The rats recovered from the lethargy and decreased reflexes 9 days post-immunosuppression.

On day 7, a day after the first fungal inoculation, sneezing and nose scratching were observed in the *Aspergillus*-infected groups and these increased with subsequent fungal inoculations.

### Interleukin 6 and 10 levels and ratio:

The pro-inflammatory cytokine (IL-6) value was higher for rats in group C (infected but untreated) compared with rats in other groups ( $p=1.34 > 0.05$ ), but this value only reached significant level ( $p < 0.05$ ) when compared with rats in treatment group D ( $p=0.018$ ),

group E ( $p=0.046$ ), and group F ( $p=0.049$ ), but not with rats in treatment group G ( $p=0.704$ ) (Table 2). Hydroxychloroquine treatment caused a decrease in IL-6 for rats in group D (infected but treated with low dose HCQ) compared with rats in other treatment groups ( $p=1.34 > 0.05$ ) but this decrease was significant when compared with rats in only treatment group G ( $p=0.041$ ), while at moderate (group E) and high (group F) dosages of HCQ (towards the LD<sub>50</sub>), the decrease in IL-6 was minimal (not statistically significant), although this minimal decrease could be attributed to oxidative stress produced by moderate/high dose of HCQ.

A higher IL-10 value was observed for rats in group G compared with rats in the other groups, but this did not reach a significant level ( $F=0.969$ ,  $p=0.470$ ). There was a direct correlation between IL-6 and IL-10 ( $r=0.649$ ,  $p < 0.001$ ). Since group C had a higher IL6/IL-10 ratio compared with other groups (Fig 1), it could be argued that group C rats had the worst outcome ( $p > 0.05$ ).

### Dysregulation of liver enzymes:

There was a direct relationship between AST and ALT ( $r=0.565$ ,  $p=0.002$ ), AST and ALP ( $r=0.391$ ,  $p=0.044$ ), and ALT and ALP ( $r=0.588$ ,  $p=0.001$ ). A higher AST was observed in group B, treated with HCQ alone, compared with the neutral control (group A-uninfected and untreated) ( $p=0.029 < 0.05$ ) (Table 2). This suggests that HCQ can induce hepatotoxicity in animals that have no underlying disease. However, treatment with HCQ among rats with aspergillosis (groups D, E and F) resulted in reduced AST levels compared with the infected and untreated group (group C) although the AST levels were not significantly different between the groups ( $p > 0.05$ ).

In group B, a higher level of ALT was also observed compared with other groups ( $p < 0.05$ ) except group C, which had the highest ALT value. The highest levels of both ALT ( $p=0.000$ ) and ALP ( $p=0.077$ ) were observed in group C rats (infected but untreated), compared to the control and treatment groups. Furthermore, infected rats treated with moderate dose of HCQ and zinc had significantly lower levels of ALP compared to other groups ( $p < 0.05$ ), except for group A, which had comparative ALP levels ( $p=0.498 > 0.05$ ). This also suggests that the addition of zinc to the treatment regime mitigated the hepatic damage induced by aspergillosis and moderate doses of HCQ.

Table 2: Mean comparison of interleukins and liver enzyme levels across experimental groups

Groups	IL-6 (pg/ml)	IL-10 (pg/ml)	AST (U/L)	ALT (U/L)	ALP (U/L)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Group A	4.41 ± 0.78	112.92 ± 24.04	10.50 ± 1.29	9.50 ± 2.38	54.25 ±19.52
Group B	4.58 ± 0.30	109.34 ± 14.82	13.75 ± 1.26	12.50 ± 0.58	71.75 ±19.52
Group C	5.11 ± 0.36	115.37 ± 12.30	12.50 ± 4.51	15.75 ± 2.50	104.50 ± 33.73
Group D	3.61 ± 1.18	101.23 ± 24.39	9.75 ± 2.06	8.75 ± 0.96	81.50 ±21.30
Group E	3.87 ± 0.56	102.24 ± 10.94	10.25 ± 0.96	10.00 ± 2.31	65.50 ±9.88
Group F	3.89 ± 0.51	116.3 ± 3.63	9.75 ± 2.06	9.75 ± 1.71	80.00 ±23.51
Group G	4.88 ± 1.40	127.92 ± 27.26	11.00 ± 1.15	9.50 ± 1.29	64.75 ±15.73
F-value	1.870	0.969	1.864	7.422	2.258
P-value	0.134	0.470	0.135	0.000*	0.077
A vs B	0.777	0.788	0.050 <sup>a</sup>	0.029 <sup>a</sup>	0.264
A vs C	0.248	0.854	0.215	0.000 <sup>c</sup>	0.003 <sup>b</sup>
A vs G	0.432	0.267	0.753	1.000	0.498
A vs D	0.182	0.384	0.637	0.565	0.088
A vs E	0.359	0.426	0.875	0.701	0.469
A vs F	0.376	0.799	0.637	0.848	0.106
B vs C	0.378	0.652	0.434	0.019 <sup>a</sup>	0.043 <sup>a</sup>
B vs D	0.110	0.544	0.018 <sup>a</sup>	0.008 <sup>b</sup>	0.529
B vs E	0.234	0.595	0.036 <sup>a</sup>	0.065	0.686
B vs F	0.247	0.602	0.018 <sup>a</sup>	0.044 <sup>a</sup>	0.594
B vs G	0.612	0.172	0.094	0.029 <sup>a</sup>	0.651
C vs D	0.018 <sup>a</sup>	0.294	0.094	0.094	0.146
C vs E	0.046 <sup>a</sup>	0.330	0.166	0.166	0.018 <sup>a</sup>
C vs F	0.049 <sup>a</sup>	0.944	0.094	0.094	0.123
C vs G	0.704	0.350	0.349	0.349	0.016 <sup>a</sup>
D vs G	0.041 <sup>a</sup>	0.055	0.434	0.434	0.284
D vs E	0.663	0.939	0.753	0.291	0.306
D vs F	0.639	0.264	1.000	0.393	0.923
E vs F	0.973	0.297	0.753	0.710	0.352
E vs G	0.096	0.064	0.637	0.836	0.961
F vs G	0.103	0.387	0.434	0.868	0.328

Statistical analysis: ANOVA and Post hoc Test. \*Significance is set at  $p \leq 0.05 = ^a$ ,  $\leq 0.01 = ^b$ ,  $\leq 0.001 = ^c$  Number of animals per group = 4.

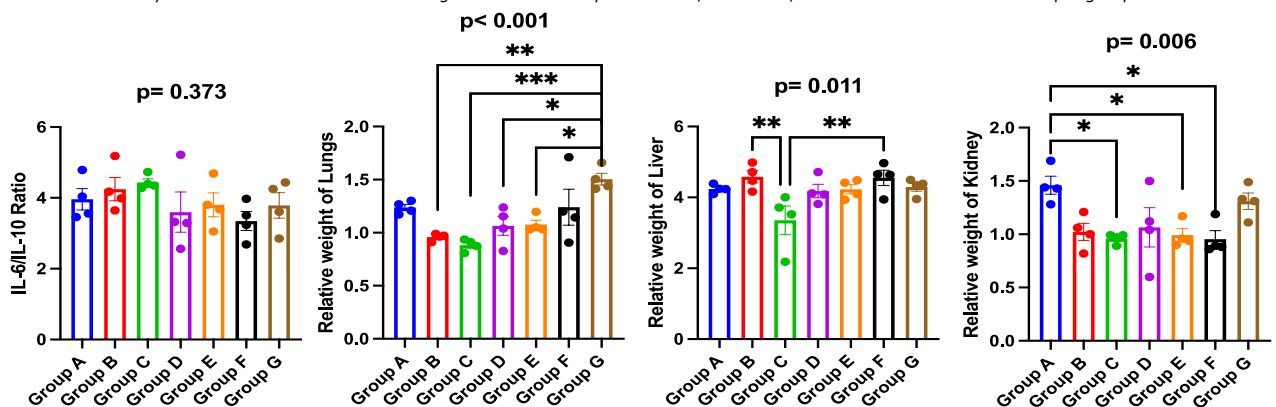


Fig 1: Relative organ weight and Interleukin ratio across experimental groups

In Fig I, rats in group C (infected but untreated) had a higher IL-6/IL-10 ratio (but this is not statistically significant at  $p=0.373$ ) and lower relative weight of the lungs, liver, and kidneys (consistent with atrophy) compared with the control and treatment groups.

The IL-6/IL-10 ratio and relative org-

an weight of Group G rats (infected but treated with moderate dose of HCQ and zinc) were like those of Group A rats (uninfected and untreated), which suggests that addition of zinc to moderate dose of HCQ improved treatment outcomes in infected rats.

Table 3: Mean comparison of renal function parameters across the experiment groups

Groups	Electrolytes (mEq/L)				Creatinine (mg/dl)	Urea (mg/dl)
	Potassium	Sodium	Chloride	Bicarbonate		
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
Group A	4.83 ± 0.25	141.75 ± 3.30	100.50 ± 4.12	24.25 ± 1.26	9.25 ± 1.26	42.00 ± 8.76
Group B	4.40 ± 0.59	142.00 ± 1.41	103.25 ± 0.96	23.25 ± 1.71	9.75 ± 0.50	44.75 ± 4.79
Group C	5.18 ± 0.83	141.50 ± 2.38	101.25 ± 1.50	25.00 ± 0.82	14.75 ± 2.22	132.25 ± 34.33
Group D	4.28 ± 0.34	145.00 ± 1.41	104.00 ± 1.41	25.00 ± 0.96	8.50 ± 1.29	49.00 ± 2.83
Group E	4.63 ± 0.43	142.50 ± 2.65	102.75 ± 2.50	23.75 ± 1.71	9.50 ± 0.58	44.25 ± 6.50
Group F	4.80 ± 0.64	143.25 ± 3.10	103.75 ± 2.06	24.50 ± 1.29	8.75 ± 0.96	45.50 ± 9.25
Group G	4.95 ± 0.39	142.50 ± 1.73	102.50 ± 1.29	24.75 ± 0.96	8.75 ± 1.50	49.25 ± 10.01
F-value	1.388	0.985	1.354	1.201	11.252	19.873
p-value	0.265	0.460	0.278	0.344	0.000*	0.000*
A vs C	0.362	0.884	0.637	0.419	0.000 <sup>c</sup>	0.000 <sup>c</sup>
A vs D	0.158	0.069	0.036 <sup>a</sup>	0.284	0.425	0.508
A vs F	0.948	0.386	0.050 <sup>a</sup>	0.786	0.594	0.740
B vs C	0.052	0.771	0.215	0.068	0.000 <sup>c</sup>	0.000 <sup>c</sup>
B vs D	0.742	0.091	0.637	0.039 <sup>a</sup>	0.190	0.687
C vs D	0.026 <sup>a</sup>	0.051	0.094	0.786	0.000 <sup>c</sup>	0.000 <sup>c</sup>
C vs E	0.158	0.561	0.349	0.184	0.000 <sup>c</sup>	0.000 <sup>c</sup>
C vs F	0.329	0.313	0.125	0.588	0.000 <sup>c</sup>	0.000 <sup>c</sup>
C vs G	0.555	0.561	0.434	0.786	0.000 <sup>c</sup>	0.000 <sup>c</sup>

Statistical analysis: ANOVA and Post hoc Test. \*Significance is set at  $p \leq 0.05 = a$ ,  $\leq 0.01 = b$ ,  $\leq 0.001 = c$  Number of animals per group = 4.

#### Alterations in renal function of rats:

Higher levels of serum creatinine and urea were observed in group C rats (infected but untreated) compared to rats in the treatment groups ( $p < 0.05$ ) (Table 3), which suggests that aspergillosis induces renal damage as well. The result in Table 3 suggests that a low dose of HCQ reduced the elevated level of potassium induced by aspergillosis in the Group D rats ( $p < 0.05$ ). A dose-dependent increase in potassium levels was also observed among rats treated with low, moderate, and high doses of HCQ ( $p > 0.05$ ).

Rats that received moderate dose of HCQ had better renal function compared to rats that received low or high doses of HCQ. Results indicate that adding zinc to moderate dose of HCQ did not improve renal function compared to treatment with HCQ alone. Pearson's correlation revealed a direct relationship between potassium and urea ( $r = 0.481$ ,  $p = 0.011$ ), sodium and chloride ( $r = 0.864$ ,  $p < 0.001$ ), and urea and creatinine ( $r = 0.700$ ,  $p < 0.001$ ).

#### Discussion:

This study assessed the immunological and biochemical effects of HCQ and zinc therapy in experimentally induced invasive

pulmonary aspergillosis. Inflammatory cytokines, especially IL-6, are important biomarkers for distinguishing trauma or inflammation associated with infections caused by pathogens such as *Aspergillus* (8,15,16). This study revealed a lower IL-6 level and improved glomerular filtration rate in *A. fumigatus*-infected groups (D, E, and F) treated with HCQ or combined therapy of HCQ and zinc, compared to *A. fumigatus*-infected but untreated group (group C). This finding suggests that HCQ possess immunosuppressive effects and could be used in the management of many inflammatory diseases.

Studies have shown that, to restore homeostasis, the release of pro-inflammatory cytokines (primarily IL-6) results in subsequent release of anti-inflammatory cytokines such as IL-10 (15,17). However, the compensatory increase in IL-10 levels is often ineffective in counteracting increasing levels of IL-6 (18). This could be the explanation for the sustained elevation of IL-6 levels despite high IL-10 levels in Group C rats in our study. Interestingly, Feng et al., (19) reported a consistent rise in serum IL-6, IL-10 and white blood cells in mild sepsis, and severe sepsis to septic shock (19). This systemic inflammatory response syndrome depicts the host acute phase immune reaction to infec-

tion and trauma, which could lead to multiple organ dysfunctions due to cytokine storm and neutrophil activation (20).

Trauma-related elevation of pro-inflammatory cytokines have been associated with respiratory distress syndrome and organ failure (21). Immune cells and cytokines play crucial roles in the pathogenesis of disease and treatment outcomes. In recent years, the ratio of pro-inflammatory (IL-6) to anti-inflammatory cytokine (IL-10) i. e. IL-6/IL-10 ratio has been used as a reliable marker of inflammation (21,22). The IL-6/IL-10 ratio was higher in groups B and C rats causing them to have low organ weight and body mass in relationship to the other groups. An increase in IL-6/IL-10 ratio has been shown to be directly associated with a poor outcome (21). Our study revealed that the IL-6/IL-10 ratio decreased significantly in group F rats, which correlated with a decrease in oxidative stress and organ damage. This is evidenced by a reduction in IL-6 level and increase in IL-10 level, especially in treatment Groups D and E. This elevation in IL-10 serves to suppress immune responses, warding off autoimmunity, and decreasing oxidative stress levels (23).

Upon the administration of zinc as an immune booster (Group G rats), there was an increase in the level of IL-6, irrespective of the synergized effects of the HCQ (which acted as an immunosuppressant in aspergillosis-induced groups). Zinc induces monocytes to produce interleukin-1 (IL-1), IL-6 and tumour necrosis factor- $\alpha$  (24). Similarly, decreased production of  $T_{H1}$  cytokines and interferon- $\alpha$  (IFN- $\alpha$ ) by leukocytes in healthy elderly persons is correlated with low zinc serum levels. It is important to note that in response to zinc supplementation, plasma cytokines exhibit a dose-dependent response (25). According to the findings of this study, the use of HCQ in the absence of aspergillosis (group B negative control) resulted in an augmentation of organ weight and elevated liver enzymes. The shift in liver function could potentially be attributed to heightened levels of IL-6.

Research indicates that IL-6 may substantially impact B cell hyperactivity and directly impact tissue harm (26). However, other research showed that HCQ was able to ameliorate aspergillosis-induced hepatotoxicity through concomitant reduction of IL-6 levels and decreased polymorphonuclear infiltrates (27). Although zinc supplements have not been shown to result in higher expression of IL-10 (28), the results from our study revealed higher expression of IL-10 when HCQ and zinc were simultaneously administered to the experimental rats. Despite the fact that HCQ has shown promise in treating respiratory diseases, there are concerns about its

potential side effects such as renal toxicity and heart failure, particularly when taken at high doses, as noted by Alanagreh et al., (29). Nevertheless, the finding of our study suggests that low dose of HCQ or combination of moderate dose of HCQ and zinc may improve renal function when dealing with aspergillosis. This finding is reinforced by a previous study that highlighted the potential beneficial effects of HCQ on renal dysfunction, as outlined by Bourke et al., (30). Taken together, it could be argued that other immunological factors may be associated with the drop in IL-6 in the HCQ treatment groups. This warrants future studies.

## Conclusion:

Our research revealed that both HCQ and zinc have the potential to alleviate cytokine levels and relative organ weight. This indicates that HCQ might be able to lower the risk of autoimmunity and systemic inflammatory response syndrome associated with systemic fungi infection such as invasive aspergillosis that can be life-threatening.

## Contributions of authors:

JOO conceptualized the research and was involved in data analysis and original manuscript draft preparation; ATB conceptualized the research and was involved in literature searches, visualization and data curation; OGO and POA were involved in literature searches, visualization and data curation; POA and NEE cultured and isolated *A. fumigatus* for inoculation in the rat models. All authors read and approved the final version of the manuscript.

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