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Haematological Parameters of rats Administered with Fungal isolates from Mosses and Ferns in Enugu Metropolis

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ABSTRACT

Fungi are spore-forming, non-chlorophytic, eukaryotic (cells having true nuclei) organisms and most of the true fungi are filamentous and branched. Most of the over 100,000 species of fungi are saprophytes. However, over 20,000 species of fungi are parasites and cause disease in crops and plants. Fungi parasites are by far the most prevalent plant pathogenic organism. Fungal pathogen are present throughout the environment, human exposure is therefore inevitable. The aim of this research was to determine the Haematological Parameters of rats Administered with Fungal isolates from Mosses and Ferns in Enugu Metropolis. Following the administration of the fungi, the lymphocyte count was consistently and significantly lower among rats that received the fungi via intraperitoneal rout compared to the intranasal route and that of the control group ($p<0.05$). Neutrophil count, basophils, monocytes and WBC were consistently higher among rats administered with fungi (*Pichia kudriavzevii, Aspergillus fumigatus, and Aspergillus flavus*) intraperitoneally.
Keywords: Haematological Parameters, rats, Fungal, Mosses and Ferns.

INTRODUCTION

Fungi are a group of non-photosynthetic microorganisms which live as saprophytes in the soil and on dead organic matter or as parasites of plants and animals including man [1,2,3,4,5]. They can be seen in any habitat but majority live on land, mainly in soil or on plant material [6,7,8,9,10]. With reference to [11,12], they are heterotrophic organisms i.e they are dependent on living organisms for their growth. They can quickly colonize all kinds of dead organic materials and are together with bacteria the most important organisms that recycle organic matter [13,14]. Most fungi are microscopic multicellular filaments consisting of septate and non septate hyphae while a few are unicellular in the form of yeast. Fungi are ubiquitous in nature; found in soil, decaying vegetation, in the air and in the water [15,16]. Sporulation is the principal means by which fungi reproduce and are dispersed through the environment [17,18,19]. The fungal spores are unicellular or multicellular reproductive units or distributional cells developing into a number of different phases of the complex life cycle of fungi [20]. They are metabolically dormant protected cells released by the mycelium in enormous numbers and are important as human allergenic antigens.They move between habitats by dispersing small spores through the atmosphere [21]. Although fungi are seen worldwide, only a few of them are considered as pathogenic causing human and animal infections [22]. Fungi are classified into four (4) phyla; Chytridiomycota, Zygomycota, Ascomycota and Basidiomycota [23]. The largest phylum is the Ascomycota which includes more than 60% of known fungi and about 85% of the human pathogens. Some mycologist defined fungi as one of the most diverse microorganisms that live in different environmental sources such as plant parts (leaves, root and fruits), soil, food and water sources [24,25]. The growth and distribution of fungi are highly influenced by some environmental factors such as temperature, pH, moisture, and degree of aeration, amount and type of

nutrients. A fungus is a eukaryote that digests food externally and absorbs nutrients directly through a substance in their cell walls known as chitin [8]. Most fungi reproduce by spores and have a body (thallus) composed of microscopic tubular cells called hyphae. Fungi are heterotrophs and like animals, obtain their carbon and energy from other organisms. Some fungi obtain their nutrients from a living host (plant or animal) and are called biotrophs; others obtain their nutrients from dead plants or animals and are called saprotrophs (saprophytes and saprobes). Some fungi infect a living host, but kill host cells in order to obtain their nutrients; these types are called necrotrophs [9].

Objective of the study

To determine the Haematological Parameters of rats Administered with Fungal isolates from Mosses and Ferns in Enugu Metropolis.

MATERIALS AND METHODS

STUDY AREA

The study was carried out in the Microbiology laboratory of Enugu State University of Science and Technology (ESUT), Enugu, Nigeria. ESUT is a major tertiary institution in Enugu located at 6°51′24″N 7°23′45″E with a land size of 500 hectares (1,200 acres).

STUDY DESIGN

This was a cross-sectional study that employed random sampling to collect environmental mosses and ferns samples as well as skin and nose swabs from recruited participants.

Identification and Collection of Mosses and Ferns

The mosses and ferns specimens were properly identified and authenticated by the curator in Applied Biology in Enugu State University of Science and Technology (ESUT).Department. The specimens were then transported to the Microbiology Laboratory of Enugu State University of Science and Technology (ESUT), in separate sterile plastic bags for fungal analysis. Fresh mosses and ferns were used for fungal isolation to reduce the chance of contamination.

Collection of skin and Nose swabs

Skin and nose swabs were collected from areas where these mosses and ferns were. 200 participants from different locations in Enugu metropolis samples were used. The samples were also transported to the laboratory for fungal analysis.

Media preparation

Potato dextrose agar (SIGMA) and sabourand was used for the isolation of fungi, and was prepared following the manufacturer's instructions, in which 42 g of potato dextrose agar (PDA) was weighed and distilled water added to make 1000ml. The medium was autoclaved at 121 °C for 15 minutes, allowed to cool to about 50 °C before the addition of 0.1 g/L of streptomycin. The mixture was stirred by gently swirling the flask, before pouring into 9 mm diameter sterile Petri dishes. The medium was allowed to solidify before inoculation.

ISOLATION OF FUNGI

Isolation of fungi was based on the method described by [8]. The mosses and ferns were washed with running tap water to remove dust and debris adhering to them. One gram (1g) of each sample (mosses or ferns) was dispensed into a prepared 9ml of distilled water contained in the McCartney bottles. The content was shaken for homogenous mixture with centrifuge and the supernatant inoculated onto freshly prepared plates of Potato Dextrose Agar (PDA) which also contains Streptomycin to avoid growth of unwanted bacteria. The plates were incubated at room temperature for 2-5 days. Following incubation, plates were examined for fungal growth.

Microscopic Examination of Fungal isolates

The fungal morphology was studied macroscopically by viewing the colony features (color, shape, size and hyphae), and microscopically with the aid of a compound microscope using lactophenol cotton blue stained slide mounted with a little portion of the mycelium [8]. Lactophenol cotton blue mount was carried out on fungal culture for full identification based on the type, pigmentation, size, shape, surface features of conidia and hyphal arrangements. Sterile mounting needles were used to carry small culture/agar block and placed onto a drop of lactophenol cotton blue on a clean slide. The agar block was then teased gently into small pieces with the two (2) sterile teasing needles (flamed intermittently over a spirit flame) for separation of the fungal elements from the agar block while maintain the integrity of the spores. Another drop of lactophenol was added and then the slide was superimposed with a coverslip avoiding air traps, pressed down gently and warmed gently over the spirit lamp to allow for effective penetration of dye in order to give good contrast. Observation under low and high power objective lens was carried out, the observation include, searching for different features of fungi including, the hyphae, conidia, sporangiophore (reproductive structure), and identification was carried out microscopically by examining the colony.

Stock solutions of antifungal agents

Antifungal susceptibility testing of the isolated fungi was performed against five antifungal agents; fluconazole, ketonazole, Griseofulvin, itraconazole and Avrotrim. These antifungal agents were used to evaluate the in vitro susceptibility of the isolated fungi. Stock solutions of fluconazole, ketoconazole and avrotim were prepared in sterile distilled water. Stock solutions of water insoluble drugs, Griseofulvin and itraconazole were prepared in 100% dimethyl sulfoxide (DMSO).

PATHOGENICITY TEST

Pathogenicity of the most commonly isolated fungi during the study or known pathogenic species based on literature were tested on albino Wister rats. A total of 18 albino Wister rats procured from the animal house, College of Medicine, Enugu State University, were used for the pathogenicity testing. The rats were 6-8 weeks old and weighed between 163g-274 g before the commencement of the pathogenicity testing. The rats were categorized into 6 groups with 3 rats per group except for group 1 which contained 2rats and served as the control group. 0.6ml suspension of fungal inoculum was administered to the rats orally and peritoneally using sterile oral catheter and disposable syringe respectively. Some of the isolated fungi including *Pichia kudriavzevii, Aspergillus fumigatus* and *Aspergillus flavus* were used for the pathogenicity testing and were inoculated nasally and intraperitoneally. The animals in the test groups were repeatedly exposed to fungal suspensions while those in the control group were only fed with rat food and water. The animals were observed closely and any behavioral change was noted. After twenty one days of administration, blood samples were collected using EDTA bottles. This sample was used for the estimation of haematological parameters such as total WBC count, PCV, differential leucocyte count etc. This was carried out using the automated haematology analyser. The rats were further sacrificed after 30 days using chloroform soaked in cotton wool in a desiccator. The liver, kidneys, stomach, intestines and lungs were harvested and preserved in 10% buffered formalin for histopathological examination.

COMPLETE BLOOD COUNT

Full blood count was analyzed using Haematology Auto Analyzer Sysmex KX-2 1N (Five part Differential). The procedure for the analysis was followed as indicated in the manufacturer's user manual and Blood film was made and stained using Leishman's Stain for morphological study.

STATISTICAL ANALYSIS:

All the experiments were performed in triplicate.Relative Frequency tells the proportion or percentage of the sample observations in each organism.

· Anova (one –way analysis of variance was for the analysis of data and mean were compared to test hypotheses about multiple sample mean of fungal isolated across different zones: Basically, testing groups to see if there is a difference between them.

· A p-value higher than 0.05 (> 0.05) is not statiscally significant and indicates strong evidence for the hypothesis. This means we retain the null hypothesis and reject the alternatives hypothesis.

RESULTS

TABLE 1: HAEMATOLOGICAL PARAMETERS

Administered fungi	Treatment groups	HAEMATOLOGICAL PARAMETERS						
		Lymphocytes Mean ± SD	Neutrophils Mean ± SD	Eosinophils Mean ± SD	Basophils Mean ± SD	Monocytes Mean ± SD	PCV Mean ± SD	WBC Count (x 10^6 mm^3)
	Control	76 ± 0.8	21 ± 0.3	3 ± 0.5	1 ± 0.1	2 ± 0.6	44 ± 0.2	12.3 ± 1.5
Pichia kudriazevii	Intraperitoneal	24 ± 0.6^{ab}	75 ± 1.4^{ab}	1 ± 0.3^{a}	0 ± 0.0	0 ± 0.0	33 ± 0.1^{a}	13.0 ± 1.4^{b}
	Intranasal	36 ± 0.7	59 ± 0.5	3 ± 0.2	0 ± 0.0	0 ± 0.0	32 ± 0.5	11.4 ± 1.8
Aspergillus fumigatus	Intraperitoneal	28 ± 0.4^{a}	76 ± 0.2^{ab}	3 ± 0.6^{b}	2 ± 0.2	2 ± 0.2	41 ± 0.6^{b}	13.5 ± 1.6
	Intranasal	31 ± 0.3^{b}	72 ± 0.6	2± 0.2	1 ± 0.1	1 ± 0.3	32 ± 0.4	12.8± 1.2
Aspergillus flavus	Intraperitoneal	27± 1.4^{ab}	67 ± 0.5^{ab}	3 ± 0.5^{b}	1 ± 0.2	2 ± 0.1	43 ± 0.3	12.9 ± 0.8^{b}
	Intranasal	35 ± 0.2	61 ± 1.1	2 ± 0.5	0 ± 0.0	1 ± 0.2	35 ± 1.0	14.1 ± 0.6

RESULTS AND DISCUSSION

Haematological Parameters of rats Administered with Fungal isolates

Mean and standard deviation of the haematological parameters of Wister rats administered with some of the isolated fungi are shown in table 1. Following the administration of the fungi, the lymphocyte count was consistently and significantly lower among rats that received the fungi via intraperitoneal rout compared to the intranasal route and that of the control group ($p<0.05$). Neutrophil count, basophils, monocytes and WBC were consistently higher among rats administered with fungi (*Pichia kudriavzevii, Aspergillus fumigatus, and Aspergillus flavus*) intraperitoneally. Eosinophil count was significantly higher among rats that received the fungi via intranasal route. See table 1 below.Evaluation of hematological indices can be employed to establish both the blood-related functions of a substance such as fungi culture filtrate or the degree of alteration or toxicological. In this study, WBC were consistently higher among rats administered with fungi (*Pichia kudriavzevii, Aspergillus fumigatus, and Aspergillus flavus*) intraperitoneally this could be indicative of inherent allergy, stress, inflammation, and infection attributable to the administration. The observations on the WBCs in this study are consistent with the reports of [7,9] on the parameters-related effects of fungi isolates on the hematopoietic systems of experimental animals.

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