



Alleviated Immunological Activities of Wistar Rat Peritoneal Neutrophils and Macrophages by Polysaccharide Rich Extract from the Fruit Bodies of *Microporus vernicipes* (Polyporales)

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Authors' contributions

This work was carried out in collaboration among all authors. Author CNM carried out the experiments. Author OM designed the experiment and wrote the manuscript. Author TC edited the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i3630984

Editor(s):

(1) Dr. Rafik Karaman, Al-Quds University, Palestine.

Reviewers:

(1) Éurica Adélia Nogueira Ribeiro, Federal University of Alagoas, Brazil.

(2) Anthony Kodzo-Grey Venyo, North Manchester General Hospital, UK.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/63601>

Original Research Article

Received 03 October 2020

Accepted 09 December 2020

Published 19 December 2020

ABSTRACT

Aim: This study aimed to assess the effect of polysaccharides from fruit body of *Microporus vernicipes* (Polyporales) on immunological activities of macrophages and neutrophils.

Methodology: Polysaccharides were extracted using hot water (PHW) and cool water (PCW) and tested on the activities of peritoneal macrophage and neutrophil in response of lipopolysaccharides. The effect of the polysaccharides was examined *In vitro* to assess the phagocytic activities by evaluating the vacuole formation, nitroblue tetrazolium dye reduction, lysosomal enzyme activity, nitric oxide, myeloperoxidase in neutrophil and on neutrophil adherence assay.

Results: The cool and hot water polysaccharides of *Microporus vernicipes* significantly improved

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the activities of macrophages and neutrophils as demonstrated by an elevated the formation of vacuole following by a high production of nitric oxide, lysosomal enzyme and superoxide. Those polysaccharides extracts of *Microporus vernicipes* also caused an increase in release of myeloperoxidase by neutrophils. Results also showed that neutrophils treated with polysaccharides extracts of *Microporus vernicipes* adhered more on a plastic surface than those untreated. It has been also found that polysaccharides of *Microporus vernicipes* may have cytotoxic effect at high concentration with IC₅₀ of 2.28 and 8.15 mg/mL for PHW and PCW respectively.

Conclusion: The result of this study indicates the immunostimulatory activity of polysaccharides of *Microporus vernicipes* by stimulating activities of macrophages and neutrophils.

Keywords: Neutrophils; macrophages; immunomodulation; polysaccharides; *Microporus vernicipes*.

1. INTRODUCTION

Mushrooms have been used for hundreds of years as medicinal resources in African countries. Recently, numerous researches on immunotherapy focused on the naturally occurring substances which exhibit beneficial therapeutic properties through stimulating the immune response mechanism of the host [1-3]. Amongst the bioactive substances targeted in various studies, polysaccharides and polysaccharide-protein complex derived from fungi/mushrooms have attracted the most attention as immunomodulators. Immunomodulating activities of mushroom polysaccharides or polysaccharide-protein complex have been associated with their effects on both the innate and adaptive immunity [4,5].

In practice, there are numerous data showing the investigation of physiological and ecological importance of fungal polysaccharides. Furthermore, polysaccharides may have potential industrial applications. The examples are polysaccharides produced by mushrooms such as *Ganoderma lucidum* [6] *Lentinula edodes* [7], *Agaricus blazei* [8], *Antrodia camphorate* [9] and *Grifola frondosa* [10] that have been presented to stimulate immune activity. Polysaccharides of a medicinal mushroom, *Microporus vernicipes* have therefore paid our attention.

Microporus vernicipes belongs to the Polyporales family and distributes in the tropical and subtropical zone including Cameroon [11]. It has previously demonstrated that the water extract of *Microporus vernicipes* can have multiple activities such as antibacterial [12] and antioxidant [13] effects. It is traditionally used in treatment of opportunistic infections, whose normally absents in immunocompetent individual. Since polysaccharides may be the major components in the water extract, we

hypothesized that the polysaccharides of *Microporus vernicipes* may have immune regulatory activity. This study was designed to explore the potential effects of the polysaccharides isolated from *Microporus vernicipes* on phagocytic activities of macrophages and neutrophils.

2. MATERIALS AND METHODS

2.1 Collection of Mushroom Specimen

The specimens of *Microporus vernicipes* were harvested from the forest surroundings Bambili in Tubah subdivision, North West Region areas of Cameroon. They were transported within 24 hours to the laboratory. The identity of the specimen was achieved by a mycologist, in the Department of Biological Sciences.

2.2 Preparation of Crude Polysaccharides

Extraction of crude polysaccharides was achieved as described by Honghui et al. [14] with some modifications. Collected fresh *Microporus vernicipes* fruit bodies were clearly washed with distilled water. Later, fruit bodies (200 g) were firstly extracted with sterile distilled water (750 ml) for 72 hours at laboratory temperature. The cool water extract was filtered through wattmann N^o 1 paper and the filtrate was kept at 4°C. The residue was extracted with boiling water for 2 hours for 3 times and the hot water extract was obtained by centrifugation (4000 x g for 10 min). Cool water extract and hot water extract were subjected to ethanol precipitation by adding of 4 volumes of 96 % ethanol. The precipitate was isolated and ovum-dried to give the cool water-soluble polysaccharide (PCW) and the hot water polysaccharide (PHW). The obtained yield of crude polysaccharides PCW and PHW were 0.16% and 2.66% of the fresh weight respectively. PCW and PHW were dissolved in sterile distilled water and subjected to membrane ultrafiltration with a molecular weight cut-off of

1000 Da to remove impurity with low molecular weight before use.

2.3 Animals for Peritoneal Cells Isolation

Wistar rats (5-weeks old) were raised in the animal house of the Biochemistry Department, University of Bamenda (North West Region, Cameroon). The animals were housed in a rodent facility at normal temperature with a 12 h light-dark cycle. They were provided with water and mouse chow ad libitum.

2.4 Isolation of Peritoneal Macrophages and Culture Conditions

Peritoneal macrophages and neutrophils were obtained using peritoneal lavage method [15]. Prior to isolation (12 hours for neutrophils and 92 hours for macrophages), 2 ml of 3% albumin (Sigma, Germany) had been injected intraperitoneally. Peritoneal exudates were collected in CRPMI-1640 medium 3 days later to obtain macrophages and 15 hours to obtain neutrophils from 3 rat each. Briefly, animals were sacrificed by dislocating cervical vertebrae and marinated in 75% ethanol for three min. Serum free RPMI-1640 medium was injected into the peritoneal cavity and withdrawn to collect the ascites. After centrifugation (1000 rpm, 5 min) using Model Z206-A centrifuge (Hermle Labortechnik GmbH), cells in the pellets were suspended in CRPMI-1640 and erythrocytes were lysed by hypotonic lysis. The cell numbers were determined by a Malassez hemocytometer and cell viability was tested by Trypan blue dye exclusion technique [16].

2.5 Cells Culture

Cells (5×10^6 cells/ml) were seeded on 96-well tissue culture plates (NUNC) in CRPMI-1640 (RPMI-1640 supplemented with 10% heat-inactivated (56°C, 30 min) FBS (fetal bovine serum), penicillin (100 units/ml) and streptomycin (100 units/mL)). All activity assays were performed in triplicates on 96 well microtiter (NUNC) and incubated at 37°C in a humidified incubator with 5% CO₂ atmosphere. For the toxicity assay, PCW and PHW were used at 0.34 to 11.1 mg/ml concentrations, while to evaluate their effect, PCW and PHW were used at 0.08 to 55.5 mg/ml concentrations.

2.6 Measurement of Cytotoxic Effects

The plates were incubated at 37°C for 2 h. Samples and LPS (1 µg/ml) as control were

added to the cultures at the concentrations indicated in each figure. The cultures were then incubated at 37°C for 20 h. Viability of cells was measured based on the mitochondria-dependent reduction of MTT [17]. The reduction of MTT was quantitated by measurement of the absorbance at 490 nm on the microplate spectrophotometer. The percentage (%) of cell viability was calculated by the following formula:

$$\% \text{ Viable cells} = 100 \times \frac{A_{\text{sample}}}{A_{\text{control}}} \quad (F_1)$$

The inhibitory effect of the polysaccharides was expressed as percentage of inhibition (F₂) and the IC₅₀ was determined using SPSS software.

$$\% \text{ Inhibition} = 100 \times \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \quad (F_2),$$

Where A_{control} indicates the optical density of cell cultured in medium only, A_{sample} indicates the optical density of cell treated with polysaccharides.

2.7 Vacuole Formation Assay

Vacuole Formation was assessed using neutral red [18]. In the 96-well plates (NUNC), cells were seeded in the RPMI-1640 containing the PCW and PHW. They were stimulated by addition of LPS (1 µg/ml) and incubated at 37°C, 5% CO₂ for 24 h. At end incubation time, 100 µl of neutral red in physiological saline solution (0.1%) were added to each well. The plates were incubated at 37°C for 30 min. After discarded the supernatant, each well was washed with 200 µl PBS twice, followed by addition of 100 µl cytolysate (acetic acid:anhydrous alcohol = 50:50). The color intensity of the neutral solution, which reflects the phagocytic activity, was measured at 570 nm using a microplate spectrophotometer (VersaMax). From the absorbance of the sample and that of Medium control, the effect of PCW, PHW and LPS was expressed as percentage of stimulation as follows:

$$\% \text{ stimulation} = \frac{A_{\text{sample}} - A_{\text{control}}}{A_{\text{control}}} \times 100 \quad (F_3),$$

Where A_{control} is the absorbance obtained for cells cultured in medium only.

2.8 Measurement of Concentrations of NO

Nitric oxide (NO) production was determined by assaying culture supernatants for nitrite using

Griess reagent [19]. Cells were seeded in a 96-well tissue culture plate for 24 h. After stimulated with PCW and PHW followed by stimulation with LPS for 24 h, the cell culture medium supernatants were collected. The supernatants (50 μ l) were mixed with equal volume of Griess reagent (1% Sulfanilamide in phosphoric acid 5%, and 0.1%, N-1-naphthylethylenediamine dihydrochloride in PBS) and incubated at room temperature for 10 min. The absorbance at 520 nm was measured using a microplate spectrophotometer (VersaMax). The effect of PCW and PHW expressed as percentage of stimulation was assessed by using the absorbance of the sample and that of Medium control. (F_3).

2.9 Measurement of Production of ROS

ROS production was quantified using the NBT [20]. Cells were seeded on 96-well tissue culture plates in RPMI-1640 containing the PCW and PHW and LPS (1 μ g/ml) as stimulant. They were incubated for 24 h followed the NBT assay. A hundred of the culture medium was incubated with 50 μ l NBT (0.33 mg/ml, diluted in PBS) in a 96-well format. The plate was incubated at room temperature, in the dark for 5 min, followed by addition of 50 μ l DMSO in each well to dissolve the purple-colored formazan crystals. The absorbance was recorded at 630 nm using the microplate reader. Cells stimulated with LPS and medium were also incubated as controls. The effect of PCW and PHW was expressed as percentage of stimulation. (F_3).

2.10 Determination of Acid Phosphatase Production

Neutrophils were incubated with PCW and PHW in the presence of LPS (1 μ g/ml). At the end of the desired length of time (24 h), and the culture media were removed. Plates were washed twice with phosphate buffer solution (PBS). The adherent monolayer was then disrupted by 0.1 ml of triton 100 (1% in PBS). Cells extracts obtained were used to assess the lysosomal acid phosphatase [18]. Cell extracts (0.1 ml) were mixed with an assay mixture containing 9.2 mmol/l para-nitrophenylphosphate (P-NPP, Sigma) and 0.1 mol/l acetate buffer (pH 6) in a final volume of 0.60 ml. The reaction was stopped by the addition of 0.15 ml 0.05 mol/l NaOH. The color was measured at 410 nm in a spectrophotometer. The effect of PCW and PHW on the amount of hydrolyzed P-NPP was then determined by using the absorbance of the

sample and that of Medium control. The enzyme activity is defined as percentage of stimulation (F_3).

2.11 Myeloperoxidase Activity Assays

Peroxidase activity was measured using 3,3',5,5'-Tetramethylbenzidine (TMB, Sigma) [20]. Briefly, 10 μ l sample were combined with 80 μ l 0.75 mM H_2O_2 (Sigma) and 110 μ l TMB solution (2.9 mM TMB in 14.5% DMSO (Sigma) and 150 mM sodium phosphate buffer at pH 5.4), and the plate was incubated at 37°C for 5 min. The reaction was stopped by adding 50 μ l 2 M H_2SO_4 (Sigma), and absorption was measured at 450 nm to estimate myeloperoxidase (MPO) activity. The effect of PCW and PHW expressed as percentage of stimulation was assessed. (F_3).

2.12 Neutrophil Adherence Assays

Aliquots of 100 μ l of PCW and PHW were added to wells of the 96-well flat-bottom culture plate, followed by the addition of an equal volume of neutrophil suspension. After agitation for 2 min, the reaction mixtures were incubated at 37 °C in a CO_2 incubator for 6 h. After incubation, the reaction mixtures in the culture plates were vortexed 40 times on a vortex mixer to remove non-adherent cells. The supernatants were immediately discarded. Thereafter, fixation and staining were performed for 30 min at room temperature by the addition to each well of 200 μ l of 0.5% crystal violet in distilled water containing 12% neutral formaldehyde solution and 10% ethanol. After being fixed and stained, the samples were thoroughly washed with tap water and the plate tapped against the sink to remove excess water. The wells were air-dried for more than 20 min. Crystal violet was extracted by addition of 100 μ l of 1% SDS (sodium dodecyl sulfate) to each well from the neutrophils that had adhered and been stained. The quantitative evaluation of neutrophil adherence to the plastic plate was performed by measuring the absorbance at 570 nm (OD value) with a microplate reader [21].

2.13 Statistical Analysis

All tests were carried out in triplicate and the results were represented as mean \pm SEM. Difference between samples and controls was analyzed by ANOVA followed by two-tailed Student's t test. Difference with $P < 0.05$ (*) or $P < 0.01$ (**) or $P < 0.001$ (***) was considered statistically significant.

3. RESULTS

3.1 Effects of Polysaccharides of *M. vermicipes* on Macrophages and Neutrophils Activities

To evaluate the activities of macrophages and neutrophils, the cells were obtained by peritoneal lavage, cultivated and treated *in vitro* with different concentrations of polysaccharides, and LPS (1 µg/ml). The effects of PHW and PCW on macrophages and neutrophils were achieved by assessing percentage of vacuoles formation, NO production, ROI production and lysosomal acid phosphatase activity. The vacuoles formation was quantified after a period of 48 hours, using the neutral red assay. The vacuolating percentage in macrophages treated with 0.69 mg/mL of PHW and 0.08, 0.17 and 0.34 mg/mL

of PCW was significantly higher ($P < 0.05$) when compared with LPS-treated or control (Fig. 1).

In neutrophils treated, the vacuolating percentage was significantly higher ($P < 0.05$) with 0.17 to 5.55 mg/mL of PHW and 0.08 to 5.55 mg/mL of PCW when compared with the results obtained in control (Fig. 1). Treatment with the PMs significantly increased ($P < 0.05$) the nitrites' production by macrophages in a concentration-dependent manner with 0.69, 1.38, 2.77 and 5.55 mg/mL of PHW and 0.08, 0.17, 0.34 and 0.68 mg/mL of PCW compared to control (Fig. 2). NO production in neutrophils was significantly higher ($P < 0.05$) when they were treated with 0.69, 1.38 and 2.77 mg/mL of PHW and 0.69, 1.38, 2.77 and 5.55 mg/mL of PCW compared to control treated with LPS only (Fig. 2).

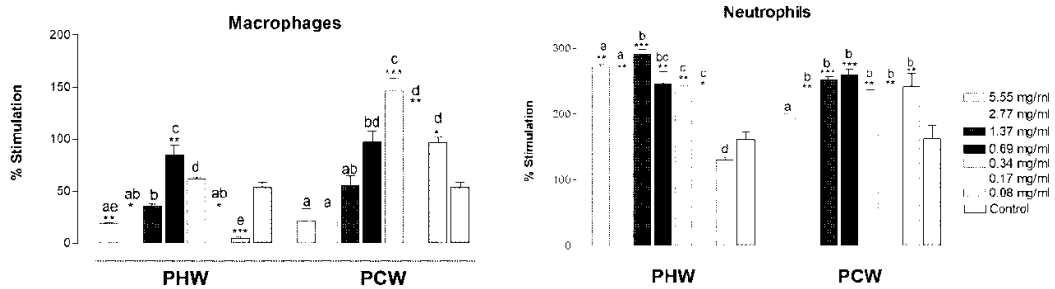


Fig. 1. Effect of the cool water (PCW) and hot water (PHW) polysaccharides of *M. vermicipes* on the macrophages and neutrophils' vacuolating formation

The results represent the mean \pm SEM ($n = 3$) and were analysed using the ANOVA test followed by Turkey's post hoc test. Asterisks indicate significant differences in comparison to control or (LPS-treated cells), with * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Letters indicate concentration dependent significant differences, with $p < 0.05$

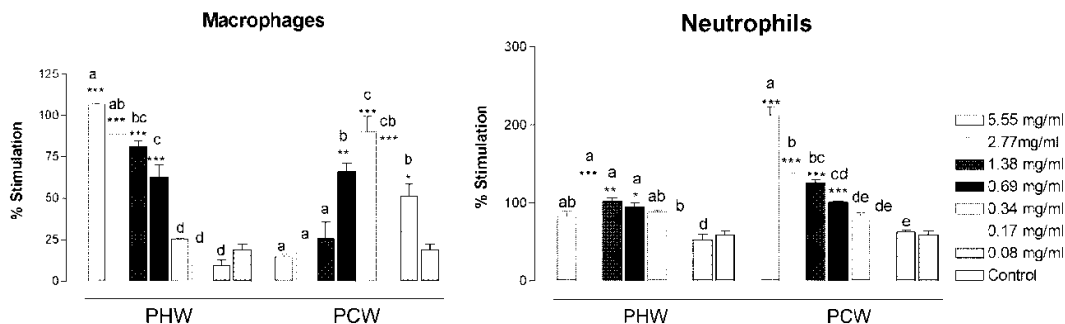


Fig. 2. Effect of the cool water (PCW) and hot water (PHW) polysaccharides of *M. vermicipes* on the macrophages and neutrophils' NO production

The results represent the mean \pm SEM ($n = 3$) and were analysed using the ANOVA test followed by Turkey's post hoc test. Asterisks indicate significant differences in comparison to control or (LPS-treated cells), with * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Letters indicate concentration dependent significant differences, with $p < 0.05$

The ROI released by macrophages cultured with 1.38, 2.77 and 5.55 mg/mL of PHW and 5.55 mg/mL of PCW from *M. vernicipes* significantly increased ($P < 0.05$) compared to control. The level of ROI released by neutrophils was significantly augmented ($P < 0.05$) by 5.55 mg/mL of PHW and PCW compared to control (Fig. 3).

mg/mL of PCW compared to control only treated with LPS.

3.2 Effects of Polysaccharides of *M. vernicipes* on MPO Activity and Adherence of Activated Neutrophils to Plastic Plates

Results show that PCW as well as PHW significantly ($P < 0.05$) increased the lysosomal acid phosphatase activity in macrophages at 0.69, 1.38, 2.77 and 5.55 mg/mL, when compared to control (Fig. 4). Also, the lysosomal acid phosphatase activity in macrophages were significantly ($P < 0.05$) increased in neutrophils treated with 0.08, 0.17, 0.34 and 0.68 mg/mL of PHW and 0.17, 0.34, 0.69, 1.38, 2.77 and 5.55

MPO activity and adherence of activated neutrophils cultivated and treated *in vitro* with different concentrations of polysaccharides was evaluated after 24 hours and 6 hours respectively. The MPO activity in neutrophils were significantly ($P < 0.05$) increased compared to the control, when treated with 2.77 and 5.55 mg/mL of PHW and 0.69, 1.38, 2.77 and 5.55 mg/mL of PCW (Fig. 5).

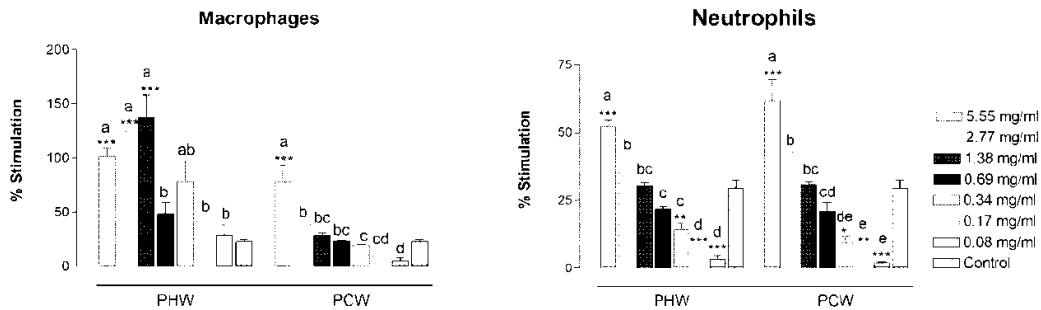


Fig. 3. Effect of the cool water (PCW) and hot water (PHW) polysaccharides of *M. vernicipes* on the macrophages and neutrophils' ROI released

The results represent the mean \pm SEM ($n = 3$) and were analysed using the ANOVA test followed by Turkey's post hoc test. Asterisks indicate significant differences in comparison to control or (LPS-treated cells), with * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Letters indicate concentration dependent significant differences, with $p < 0.05$

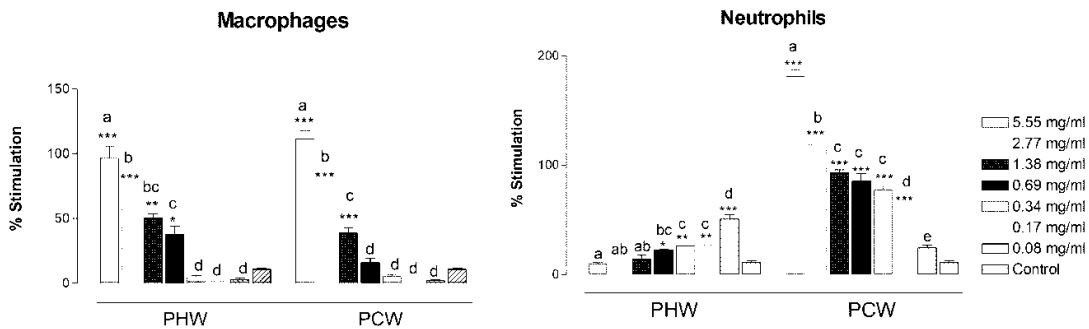


Fig. 4. Effect of the cool water (PCW) and hot water (PHW) polysaccharides of *M. vernicipes* on the macrophages and neutrophils' lysosomal acid phosphatase activity

The results represent the mean \pm SEM ($n = 3$) and were analysed using the ANOVA test followed by Turkey's post hoc test. Asterisks indicate significant differences in comparison to control or (LPS-treated cells), with * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Letters indicate concentration dependent significant differences, with $p < 0.05$

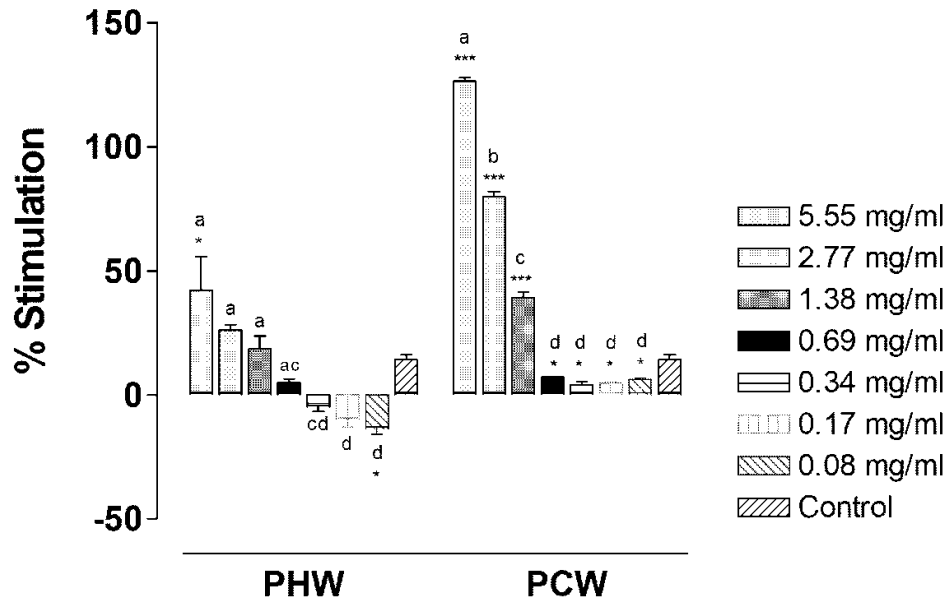


Fig. 5. Effect of the cool water (PCW) and hot water (PHW) polysaccharides of *M. vernicipes* on the macrophages and neutrophils' MPO activity

The results represent the mean ± SEM (n = 3) and were analysed using the ANOVA test followed by Turkey's post hoc test. Asterisks indicate significant differences in comparison to control or (LPS-treated cells), with *p < 0.05, **p < 0.01 and ***p < 0.001. Letters indicate concentration dependent significant differences, with p < 0.05

Results of this study demonstrate that the polysaccharides of *M. vernicipes* affect the neutrophils adherence (Table 1). The OD value of each well, which is proportional to the number of neutrophils per well, was proportional to the increasing concentration of PHW and PCW.

Compared to control (untreated neutrophils), a significant increase in OD value was detected in the neutrophil treated with PHW as well as PCW at 0.08, 0.17, 0.34, 0.69, 1.38, 2.77 and 5.55 mg/mL.

Table 1. Adherence of neutrophils stimulated with LPS and polysaccharides of *M. vernicipes* at various cell concentrations

Treatment	OD 492 nm			
	Cell concentrations (per well)			
	5x 10 ⁵		2.5x10 ⁵	
	PHW	PCW	PHW	PCW
Untreated cells	0.46±0.09	0.46±0.09	0.27±0.01	0.27±0.01
LPS 1 µg/ml	0.66±0.05*	0.66±0.05*	0.46±0.06*	0.46±0.06*
Polysaccharides (mg/ml)				
5.55	1.19±0.04***	1.70±0.13***	0.68±0.02***	0.97±0.09***
2.77	0.91±0.12*	1.40±0.06***	0.67±0.01***	0.62±0.03***
1.38	0.71±0.15	0.85±0.04**	0.58±0.04***	0.59±0.04***
0.69	0.70±0.03	0.84±0.02**	0.58±0.01***	0.58±0.01***
0.34	0.65±0.04	0.76±0.07*	0.54±0.02**	0.52±0.03**
0.17	0.59±0.03	0.64±0.07*	0.49±0.01*	0.49±0.01*
0.08	0.62±0.16	0.52±0.02	0.48±0.01*	0.46±0.01*

The results represent the mean ± SEM (n = 3) and were analysed using the ANOVA test followed by Turkey's post hoc test. Asterisks indicate significant differences in comparison to untreated cells, with *p < 0.05, **p < 0.01 and ***p < 0.001

3.3 Effects of Polysaccharides of *M. vernicipes* on Macrophages Viability

To evaluate the cytotoxic effect of polysaccharides of *M. vernicipes*, MTT assay was realized on peritoneal macrophages. The assay revealed that the extracts had inhibitory effect on the viability of peritoneal cells (Table 2). PHW as well as PCW of *M. vernicipes* exhibited a significant inhibitory effect on the macrophages viability. The IC₅₀ of PHW and PCW was 2.28 and 8.15 mg/mL respectively.

4. DISCUSSION

M. vernicipes is recommended by traditional healers to treat opportunistic infections, in immunocompetent individual such HIV positive patients. Previously, some studies have reported the antibacterial [12] and antioxidant [13] effects of this plant, but have not been evaluated its effect over the immune system. This work aimed to evaluate the immunomodulatory effect of the crude polysaccharides (PHW and PCW) from *M. vernicipes* over peritoneal neutrophil and macrophage functions. Macrophages and neutrophils play an important role in recognition of foreign antigens, homeostasis, tissue repair and immunity. Phagocytosis process represent the major function of macrophages and neutrophils as they are able to destroy pathogens in part through ROS as O₂⁻ or reactive nitrogen species (RNS) as NO [22,23] and lysosomal enzymes as acid phosphatase [24]. These free radical species, including ROS and reactive nitrogen species (RNS), are continuously generated during aerobic metabolism [25]. Estimation of ROS levels in the culture media, in addition of the assessment of phagocytic index, is therefore an important step

in understanding the phagocytes mechanisms contributing to fight invading microorganisms or to clear the body from abnormal cells.

In this study, the PHW and PCW of *M. vernicipes* upgraded the vacuolating activity in macrophages and neutrophils stimulated by LPS. The production of NO and O₂⁻ as well as the lysosomal acid phosphatase of LPS-stimulated macrophages and neutrophils also significantly increased by PHW and PCW of *M. vernicipes*. In addition, the PHW as well as PCW of *M. vernicipes* enhanced the MPO activity of the neutrophils stimulated by LPS and adherence of neutrophils in a significant concentration-dependent manner. In the scientific literature no studies have evaluated the polysaccharides of fruit bodies or some metabolites isolated from *M. vernicipes*. But, some reports have shown the immunostimulatory effect of mushroom. For example, our previous study on the aqueous extract *T. letestui* and *T. clepeatus* showed a good dose-response effect in antibody production and hypersensitivity test *in vivo* in mice [26,27]. Study carried out on various mushroom species such as *Amauroderma rude*, showed that mushrooms species can increase the immunological capacities of spleen lymphocytes, macrophages and natural killer cells as well as macrophage metabolism and antibody production [14]. Other studies on *Ganoderma lucidum* [6] *Lentinula edodes* [7], *Agaricus blazei* [8], *Antrodia camphorate* [9] and *Grifola frondosa* [10] reported that polysaccharides were one of the important bioactives responsible of immunomodulatory activities of mushroom. These findings indicate that polysaccharides of *M. vernicipes* are potential immuno-stimulator.

Table 2. Cell viability and inhibitory effect of cool water polysaccharides (PCW) and hot water polysaccharides (PHW) of *M. vernicipes*

Treatment	PHW		PCW	
	% Viable cells	% Inhibition	% Viable cells	% Inhibition
Medium	100	-	100	-
LPS -1µg/ml	129.44±5.42 ^{***}	0	129.44±5.42 ^{***}	0
Polysaccharides (mg/ml)				
11.1	39.93±2.76 ^{***}	60.06	47.17±3.14 ^{***}	52.82
5.55	45.94±3.03 ^{***}	54.05	50.07±2.02 ^{***}	49.92
2.77	45.42±7.40 ^{***}	54.57	51.73±8.58 ^{***}	48.26
1.38	51.31±10.14 ^{***}	48.68	56.16±5.78 ^{**}	43.83
0.69	63.43±19.30 ^{***}	36.56	64.88±26.93 [*]	35.11
0.34	66.00±1.70 ^{**}	33.99	66.81±12.13 [*]	33.18
IC ₅₀	2.28		8.15	

The results represent the mean ± SEM (n = 3) and were analysed using the ANOVA test followed by Turkey's post hoc test. Asterisks indicate significant differences in comparison to untreated cells, with *p < 0.05, **p < 0.01 and ***p < 0.001

In the immune response, secretion of NO and is mainly controlled by iNOS [28]. This enzyme, iNOS and other secretion by macrophages and neutrophils are mainly regulated by NF- κ B, MAPKs and AKT [29]. The resulted signaling mechanism would finally decide the secretion profile of macrophages [30]. In the present study, this signaling mechanism was not studied to elucidate the signal transduction, however macrophages and neutrophils were activated by LPS and this response was stimulated by polysaccharides of *M. vernicipes*. As LPS is known to activate macrophages essentially through NF- κ B [31], the activity of polysaccharides of *M. vernicipes* may result from effect on NF- κ B signaling pathway.

In our study, the PHW and PCW of *M. vernicipes* did not showed a significant cytotoxic effect in macrophages. Also, the PHW and PCW of *M. vernicipes* showed a low but significant increase in the proliferation of the macrophages in a concentration-dependent manner.

4. CONCLUSION

Polysaccharides of *M. vernicipes* showed to have strong functions in immunopotentiality *in vitro*. We found that the PHW and PCW were able to up-regulate in macrophages and neutrophils the phagocytic index, production of ROS and RNS, lysosomal acid phosphatase production, neutrophils' MPO activity and adherence.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All procedures involving animals and their care were approved by the Ethics Committee of the Biochemistry Laboratory.

ACKNOWLEDGEMENTS

All are grateful to the Institute of Agricultural Research for Development (IRAD) Bambui, Cameroon. The authors are grateful to the Director of IRAD and its collaborators. The authors are grateful to Njuonkou L-A of the Department of biological Sciences, University of Bamenda, for the identification of the mushroom specie.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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