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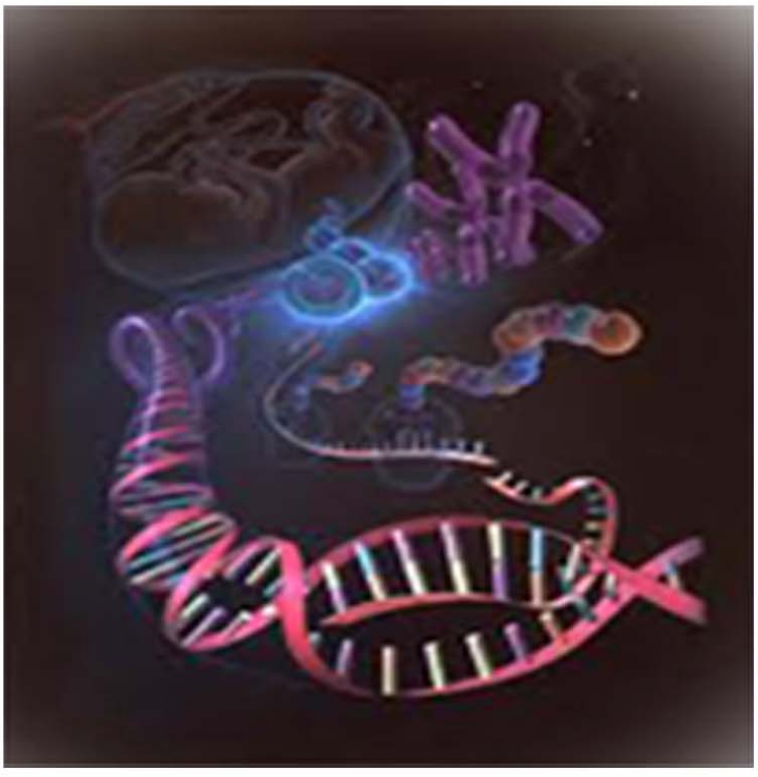

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Research Paper

CHANGES IN CYTOKINE EXPRESSION IN RATS ADMINISTERED EXTRACTS OF *TERMITOMYCES ROBUSTUS* AND *OCIMUM GRATISSIMUM*

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The study evaluated the immunomodulatory activities of extracts of *Termitomyces robostus* and *Ocimum gratissimum* on Wistar albino rats. A total of twenty Wistar albino rats were randomly grouped into five groups. The positive control was administered normal saline while the negative control received pyrogallol to suppress the immune system. Group one and two received 100 and 300 mg/kg body weight of methanol extract of *Ocimum gratissimum* and 10% pyrogallol while group three received 300 mg/kg body weight of *Termitomyces robostus* and 10% pyrogallol. The immunological activity was evaluated on cytokine expression and haemaglutination titre. The study lasted for 21 days. Significant ($p<0.05$) increases were induced by the 300 mg/kg body weight administration of both extracts on serum interleukin-2, interferon-γ, and tumor necrosis factor-α. The two doses administration of *Termitomyces robostus* resulted in significant ($p<0.05$) decrease in delayed type hypersensitivity while the administration of *Ocium gratissimum* increased ($p>0.05$) the delayed type hypersensitivity. The primary and secondary antibody titres showed significant ($p<0.05$) increase. These comparisons were made against the negative control. The results from the study demonstrate that the extracts of *Termitomyces robostus* and *Ocimum gratissimum* possess immunostimulatory effects. These effects may be the reason for their use in traditional medicine.

Keywords: Cytokines, *Termitomyces robostus, Ocimum gratissimum*

INTRODUCTION

The immune system is a network of lymphoid organs, tissues, cells and the products of these cells whose function is to defend the organism against infective agents or foreign bodies (Anaso and Onochie, 1999; Aderem and Underhill, 2009; Lam *et al.*, 2010; Egba *et al.*, 2011). The immune system adapts its response during an infection to improve its recognition of the pathogen. This improved response is then retained after the

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pathogen has been eliminated in the form of an immune memory and allows the adaptive immune system to mount faster and stronger responses (Mayer, 2006). Inappropriate immunity has been shown as a common etiology in an ever-growing array of pathological processes including infections, allergy, aging, cancer, and a variety of disorders of various organs (Tsai *et al*., 2011). Many bioactive compounds are produced by plants throughout their development for a number of reasons such as defense against microorganism, insects and herbivore (Crozier *et al*., 2006).

Ocimum gratissimum is a herbaceous plant which belongs to the *Labiate* family. The plant is used by the Ibos of Southern Nigeria in folklore medicine in the management of many health conditions (Prabhu *et al.*, 2009). Pharmacological studies on the plant have shown that it has antimicrobial and antifungal (Orafidiya *et al*., 2006; Dubey *et al*., 1997), ovicidal (Pessoa *et al*., 2002), anti diarrheal (Ilori *et al.*, 1996), wound healing (Orafidiya *et al.*, 2005) and anti-inflammatory properties (Sahouo *et al.,* 2003). Other reports have shown that it also has antimutagenic, cytotoxic and immunostimulatory effects among numerous other activities (Dubey *et al*., 1997; Obaseik-Ebor *et al.*, 1993; Oladunmoye, 2006). Our previous report showed immunostimulatory effect of the plant by the stimulation of antibody production and enhanced inflammatory response in Albino rats (Egba *et al.,* 2014).

Termitomyces robustus belongs to the family *Lyophyiceae*. They are rich in minerals such as potassium, calcium, magnesium, iron and manganese, and are mostly edible (Mattila *et al.,* 2001). The polysacharrides or glucans derived from the mushroom species have been widely recognized as immunomodulators as they are able to stimulate the host immune system and exert antitumor or antimicrobial activities along with other medicinal effects (Lindequist *et al*., 2001; Wasser, 2002). It has also been suggested that higher degree of structural variability and conformational complexity of these polymeric molecules may be responsible for the potent host immune response generation (Bohn and Bemiller, 1995). Despite the structural and functional similarities of these glucans, they differ in their ability to elicit various responses, particularly cytokine expression and production (Anderson and Stasovski, 1992). Records of health promoting benefits such as antioxidant, antimicrobial, anticancer, cholesterol lowering and immunostimulatory effects have been reported for some species of mushroom (Anderson and Stasovski, 1992; Mizuno, 1995; Mau *et al*., 2004).

Modulation of cytokine secretion may offer novel approaches in the treatment and defense of a variety of diseases. One strategy in the modulation of cytokine expression may be through the use of herbal medicines (Kevin *et al.,* 2006). Cytokines are now seen to be crucial to innate and adaptive inflammatory responses, cell growth and differentiation, cell death, angiogenesis and developmental as well as repair processes (Oppenheim, 2001). In addition, cytokines provide a link between organ systems, providing molecular cues for maintaining physiological stability (O'Sullivan *et al.*, 1998). As a result of the growing recognition of cytokine activities, altering cytokine expression and targeting their receptors may offer therapeutic potential. Current pharmacological strategies include cytokine antagonist, agonist, inhibition, and stimulation models (Sommer, 1999). There is a modest body

of knowledge on the health benefit of *Ocimum gratissimum* and *Termitomyces robustus* in human health by immunomodulation. To explore the potentially health-promoting properties of *Ocimum gratissimum* and *Termitomyces robustus*, this study evaluated their immunomodulatory effects by their cytokine expression in Albino rats.

MATERIALS AND METHODS

Plant Materials: Fresh mushroom *Termitomyces robustus* and leaves of *Ocimum gratissimum* were purchased from a local farm in Umuariaga, Ikwuano LGA of Abia State, Nigeria and were identified by Dr. Omosun of Plant Science Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Preparation of Hot Aqueous and Methanol Extracts: The dried powdered mushroom sample (10 g) was dissolved in 100 mL of hot distilled water in a beaker and left to stand overnight (12 h) with intermittent shaking and then filtered. The filtrate was concentrated in a water bath and used in the study. The hot aqueous extract of mushroom samples were continuously prepared according to demand. The methanol extract of *Ocimum gratissimum* were prepared continuously according to the demand. Also, the dried powdered leaves (150 g) of the plant were dissolved in 1500 mL of methanol in a sterile flask and swirl to ensure effective mixing and intermittent shaking. After 48 h, the mixture was decanted and solution obtained was poured into a stainless tray and allowed to evaporate to dryness at room temperature for two days using a vacuum evaporator (RC 1008 Bibby Sterilin, United Kingdom). The wet residue was freeze dried using a vacuum freezer drier and used for the study.

Preparation of 10% Pyrogallol: Pyrogallol reagent was prepared by dissolving 10 g of pyrogallol in 100 mL of distilled water. It was vigorously shaken for uniform mixing and the solution was used for the study.

Antigen Preparation: Fresh blood was collected from sheep sacrificed in a local slaughter house and preserved in EDTA bottles. It was washed three times with normal saline by centrifugation and the resulting suspension was adjusted to 1 × 10 SRBC/mL for immunization and challenge.

Experimental Design: A total of twenty male Wistar albino rats weighing between 70-110 g were randomly divided into five groups and used for the experiment. All the animals were housed in an animal house under normal conditions and acclimatized for two week. Commercial pellet diet (Vital feeds Nigeria, Ltd.) and water were fed to the animals *ad libitum.* The control groups, that is, the positive control (normal control) received normal saline via gavage while the negative control received 100 mL pyrogallol solution. Group 1 received 100 mg/kg body weight of *Ocimum gratissimum* extract and pyrogallol solution. Group 2 received 300 mg/kg body weight of *Ocimum gratissimum* and pyrogallol solution. Group 3 also received 300 mg/kg body weight aqueous hot extract of *Termtomyces robustus.* All administrations were done daily for 21 days. The protocol was approved by the experimental animal ethics committee of the College of Natural and Applied Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Sheep Red Blood Cell (SRBC) – Induced Humoral Antibody (Ha) Titer: To specifically access the effects of the extracts on antibody formation, groups of four rats per treatment were immunized with 0.1 mL of Sheep Red Blood cell

suspension (1.1×10^8 SRBC/mL) intraperitonially. The day of immunization was referred to as Day 0. Seven days later (Day 7), the rats were challenged by injecting 0.1 mL SRBC suspension into their left hind foot pad. Blood samples were collected from all the animals separately by ocular puncture using glass capillary tube on Day 7 (after challenge) for measurement of primary antibody titer and on day 14 for measurement of secondary antibody titer. Antibody levels were determined by the method described by Shinde *et al*. (1999). The collected blood was allowed to clot, and then centrifuged to get serum. 25 µL serum samples were placed into a 96-well micro titer plate. Serial two fold dilutions of the serum were made using 25 µL normal saline each time of transfer across the plate. To the 25 µL diluted serum in each well, 25 µL of 1% v/v SRBC suspension in normal saline was added. The micro titer plate was maintained at room temperature 1 h and their content examined for hemagglutination. The value of the highest serum dilution showing hemagglutination was defined as the antibody titer for the given rat.

STATISTICAL ANALYSIS

The data are expressed as mean ± standard deviation in bar charts. Comparisons were made between the groups using the one way Analysis of Variance (ANOVA) followed by post HOC LSD test. The analysis were carried out in SPSS for Windows 16. The accepted level of significance was at $p < 0.05$.

RESULTS

The effects of the administration of *Ocimum gratissimum* and *Termitomyces robustus* on interleukin-2 expression is shown in Figure 1. The results show that the administration of 300 mg/kg body weight of *Termitomyces robustus* and *Ocimum gratissimum* significantly increased the expression of interleukin-2 relative to the negative control group ($p<0.05$). Also, the administration of 100 mg/kg body weight of *Ocimum gratissimum* elicited a significant increase in the serum interleukin-2 expression ($p<0.05$). Figure 2 represents the effects of the administration of the extracts of *Ocimum gratissimum* and

Figure 1: Effects of the *Ocimum gratissimum* and *Termitomyces robustus* extracts on serum interleukin-2 (IL-2)

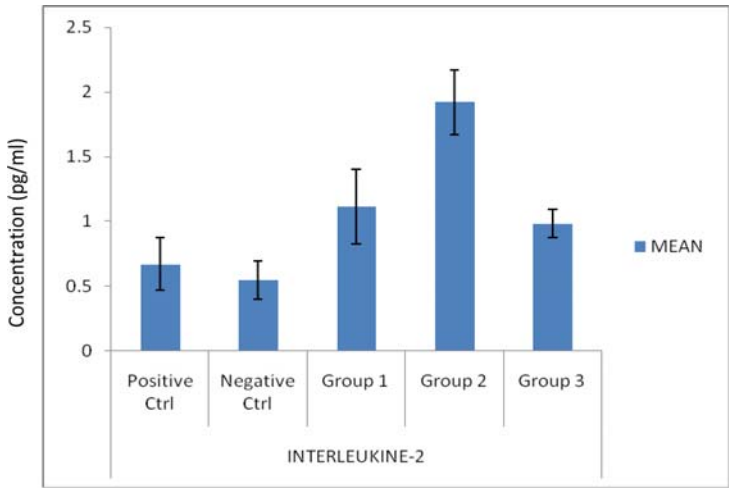


Figure 2: Effects of the *Ocimum gratissimum* and *Termitomyces robustus* Extracts on Serum Interferon-γ (IFN-γ)

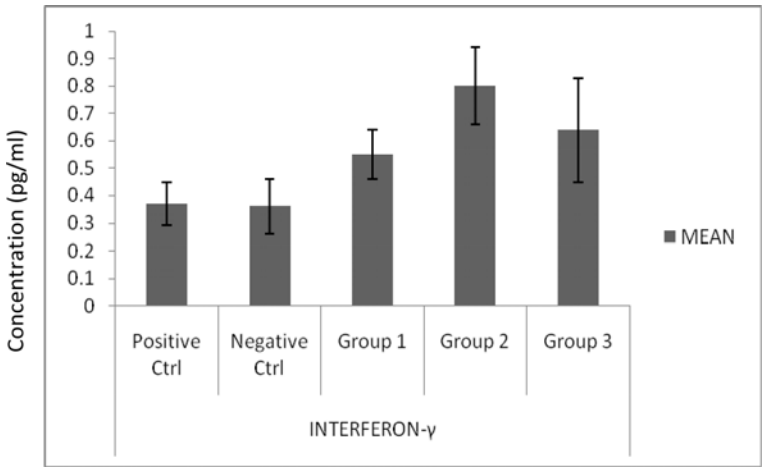
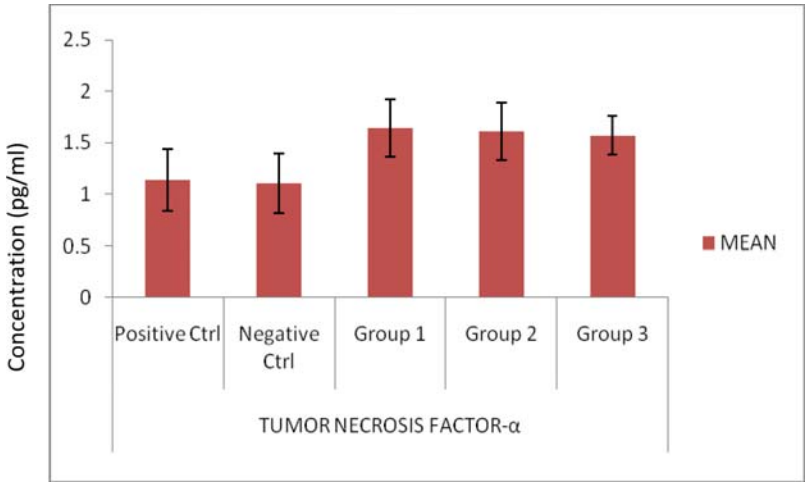


Figure 3: Effects of the *Ocimum gratissimum* and *Termitomyces robustus* extracts on serum tumor necrosis factor (TNF-γ)



Termitomyces robustus on the cellular expression of interferon-γ (IFN-γ). From the results obtained, it is apparent that the administration of 300 mg/kg body weight of *Ocimum gratissimum* produced the highest expression of interferon ($p<0.05$) when compared to the negative control. Also the administration of 300 mg/kg body weight of *Termitomyces robustus* (group 3) significantly increased ($p<0.05$) the expression of serum interferon relative to the negative control group. The results in Figure 3 represent the effects of the different extracts on tumor necrosis factor (TNF-α). The results indicate a significant increase ($p<0.05$) upon the administration of 100 and 300 mg/kg body weight of *Ocimum gratissimum* compared to the

Figure 4: Effect of *Ocimum gratissimum* and *Termitomyces robustus* extracts of on primary humoral antibody

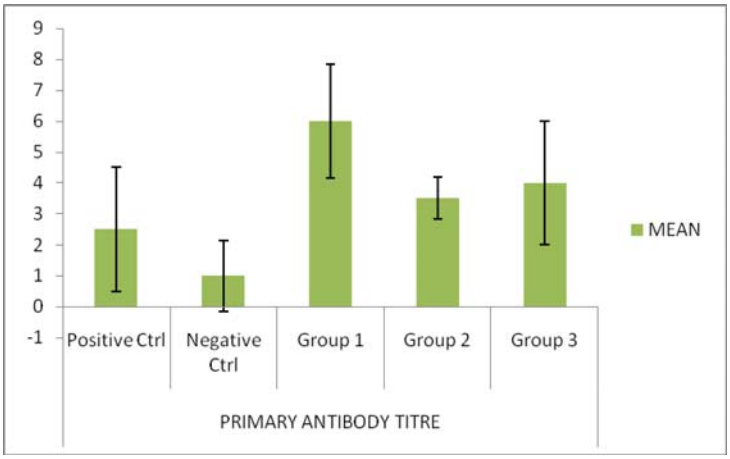
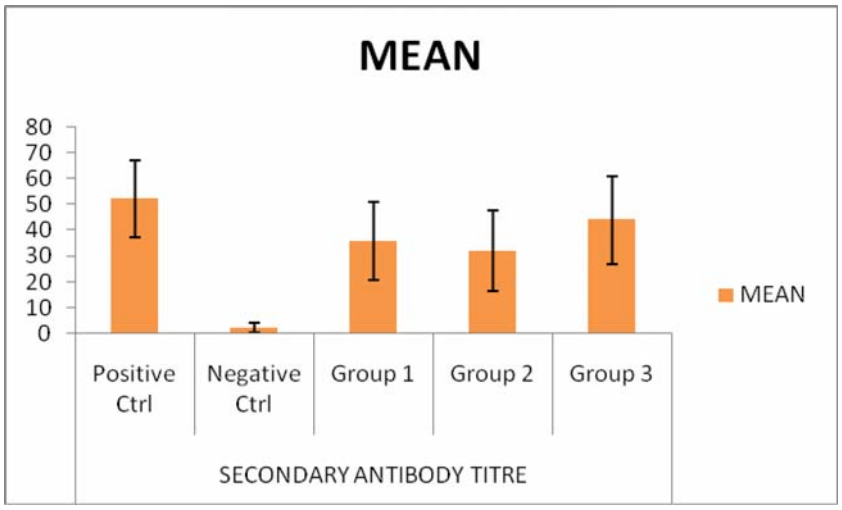


Figure5: Effect of *Ocimum gratissimum* and *Termitomyces robustus* extracts on secondary humoral antibody



negative control group. Also from the results, we observed that administration of 300 mg/kg body weight of *Termitomyces robustus* induced a significantly ($p<0.05$) increased expression of serum tumor necrosis factor (TNF-α) relative to the control.

The effects of the extracts on the production of primary antibody is shown in Figure 4. The results show that the extracts induced an increased primary antibody titre relative to the negative control group ($p<0.05$). Also, both extracts (*Ocimum gratissimum* and *Termitomyces robustus*) were able to significantly increase ($p<0.05$) the secondary humoral antibody in the rats (Figure 5).

DISCUSSION

The limitations and paucity of currently available

pharmacologic agents that have immune boosting properties have stimulated research on novel immunostimulatory and immunomodulatory agents with different mechanisms of action. Some plant products modulate both humoral and cellular immunity, while others activate just the cellular components of the immune system. Therefore, the evaluation of plants for their capacity to promote or inhibit immunocyte proliferation is necessary to the study of immunomodulation and drug discovery (Shinde *et al.*, 1999; Schneider *et al*., 2003; Smith, 1998). There are a few scientific reports on the effect of medicinal plants and mushrooms on immunity (Hardy and Chaudhri, 1997) but there is no scientific report on the effect of *Ocimum gratissimum* (Scent leaves) and *Termitomyces robustus* on cytokine expression so far. In this study, methanol extract of *Ocimum gratissimum* and hot water extract of *Termitomyces robustus* were used to examine the activity of the plant and mushroom on adaptive immunity (especially cytokine expression).

Immune response to sheep red blood cells has been widely used in the study of animal immune status (Ikeme and Adelaja, 1990). The use of Sheep Red Blood Cells (SRBC) challenge is based on the fact that responses to it is T-dependent and T-cells (T-helper cells specifically) coordinate the battle against infection by activating macrophages, B-cells and T-cells and thus, indirectly control immunoglobin production (Sherman and Halliquest, 1990). T-cells are responsible for production of cytokines. Result of this study showed the extracts significantly ($p<0.05$) stimulated antibody production in the subjects compared to rats not given any extracts (Figure 4 and 5).

The results of cytokines expression (Figure 1) showed an up regulation in the serum interleukin 2 (IL-2) expression upon the administration of 300 mg/kg body weight of the extracts of *Ocimum gratissimum* and *Termitomyces robustus* (OG and TR). It is known that IL-2 has an immune stimulating activity as it supports proliferation (Anaso and Onochie, 1999; Cornish *et al.*, 2006; Kawasaki *et al.*, 2002) and survival (Gillis *et al.*, 1977) of T cells (Gillis *et al.*, 1978). The proliferation and survival could be as a result of the ability of IL-2 to induce protein synthesis in antigen-activated T-cells. Also its absence abrogates amino acids uptake and subsequent incorporation into proteins (Mitra *et al.*, 1999).

Interferon are responsible for inducing transcription of a large group of genes which play a role in host resistance to viral infections, as well as activating key components of the innate and adaptive immune systems including antigen presentation and production of cytokines involved in activation of T cells, B cells and natural killer cells (Ngoupayo *et al*., 2009). From the results obtained, it is apparent that the administration of 300 mg/kg body weight of OG and TR induced the high expression of interferon ($p<0.05$) when compared to the negative control. These results suggest that the extracts could possess antiviral and stimulatory activities on intracellular pathogens (Egba *et al.,* 2014; Sy *et al.,* 2004). These results agree with our earlier finding that extract of *Gongronema latifolium* up regulates interferon expression in rats (Egba *et al.*, 2014).

The increase in the expression of tumor necrosis factor on both low and high dosage groups when compared against the negative control group also supports the observed influence of OG and TR on cellular immunity

(Figure 3). Tumor Necrosis Factor (TNF) super family of cytokines represents a multifunctional group of proinflammatory cytokines which activate signaling pathways for cell survival, apoptosis, inflammatory responses and cellular differentiation (Shinde *et al.*, 1999; Schneider *et al*., 2003, Krishnaraju *et al.*, 2009). Ijeh *et al.* (2004) had reported that OG contains alkaloids, tannins, phytates, flavonoids and polysaccharides. A variety of phytochemicals such as polysaccharides (Shin *et al.*, 2002) and flavonoids (Zhao *et al.,* 2007) have been reported to modulate the immune system. Flavonoids which are polyphenols have been reported to serve as immunomodulators (Shin *et al.*, 2002). Flavonoids stimulate human peripherial blood leukocyte proliferation. They also increase the activity of helper T cells, cytokines, interleukin- 2, and macrophage and are useful in the treatment of several diseases caused by immune dysfunction (Egba *et al.*, 2014). Thus, the probable mechanism of action of *Ocimum gratissimum* lies in its flavonoid component which stimulates the Helper T-cells to produce more interleukin-2 and interferon-γ. Studies have shown that mushrooms are rich sources of various bioactive molecules having anticancer and immunomodulatory potentials (Sharma *et al.,* 2012). Among the most important constituents of mushroom *Termitomyces* are certain polysaccharides known as beta-glucans, which are bound to proteins (Borchers *et al.*, 1999). Polysaccharides or beta glucans derived from mushroom species have been widely recognized as immunomodulators as they are able to stimulate the host immune system and exert antitumor or antimicrobial activities along with other medicinal effects (Lindequist *et al.*, 2005; Wasser, 2002). The polysaccharides from the mushroom *Termitomyces robustus* and other mushroom extracts do not attack cancer cells and other diseases of the immune system directly; they produce their antitumor effects by activating different immune response in the host (Wasser, 2002). These reports clearly suggest that the probable immune stimulatory mechanism of TR could be the stimulation of the helper T cells.

We reported that the extracts (OG and TR) induced a significant increase on both the primary and secondary antibody titres ($p<0.05$) between the test groups compared to the negative control group. Humoral immune response is a type of acquired immunity. B-cell activation is a large part of the humoral immune response. Upon infusion of the SRBC in the experimental rat models, the B-cells make antibodies against the soluble sheep red blood cell. The B-cells are usually activated to produce antibodies against antigens by T-cell dependent and T-cell activation. Thus the humoral antibody titres mean values, which increased in all groups except in suppressed group (negative control), was as a result of increased immune response to the foreign sheep red blood cell.

CONCLUSION

Our observations have clearly demonstrated that *Ocimum gratissimum* and *Termitomyces robustus* extracts showed considerable immunostimulatory effects on both the innate function and adaptive immune function while exhibiting proinflammatory activity as depicted by the increase in interleukine-2 expression in albino rats. Thus, the effect of the plant and mushroom on cytokine expression may be the reason for its use in traditional medicine. Further research is needed to further characterize the active bioactive agents responsible for the immunomodulatory effects of these plants.

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REFERENCES

1. Aderem A and Underhill D. M (2009), "Mechanism of Phagocytosis in Macrophages," *Annu. Rev. Immunol*, Vol. 17, pp. 593-623.
2. Anaso H U and Onochie C C (1999), "A Comparative Study of Nutrients in *Gongronema latifolium, Piper guidneense* and *Piper nigrum* to Testify High Acceptability in Local Dishes," *J. Sci. Eng. Technol*, Vol. 6, No. 20, pp. 1321-1832.
3. Anderson J B and Stasovski E (1992), "Molecular Phylogeny of Northern Hemisphere Species of *Armillaria.* Mycologia", Vol. 84, pp. 505-516.
4. Bohn J and Bemiller J (1995), " (1→3) β-Glucans as Biological Response Modifiers: A Review of Structure–functional Activity Relationships", *Carbohydr. Polymers,* Vol.28, pp. 3–14.
5. Borchers A T, Stern J S, Hackman R M, Keen C N and Gershwin M E (1999), "Mushroom, Tumors, and Immunity," *Proc. Soci. Exp. Biol. Med,* Vol. 221, No. 4, pp. 281–293.
6. Cornish G H, Sinclair L V and Cantrell D A (2006), "Differential Regulation of T-cell Growth by IL-2 and IL-1 Blood," Vol. 108, pp. 600-608.
7. Crozier A, Jaganath I B and Clifford M N (2006), In: Crozier A, Clifford MN and Ashihara H (Eds.), *Plant Secondary Metabolites: Occurrence, Structure, and Role in the Human Diet,* Blackwell Publishing, Oxford, pp. 1-24.
8. Dubey N K, Kishore N, Varma J and Lee S Y (1997), "Cytotoxicity of the Essential Oils of *Cymbopogon citratus* and *Ocimum gratissimum*", *Indian J. Pharm. Sci,* Vol.59, pp. 263-4.
9. Egba S I, Omeoga H C and Njoku O U (2014), "Oral Administration of Methanol Extract of *Gongronema latifolium* (utazi) Up-regulates Cytokine Expression and Influences the Immune System in Wistar Albino Rats", *World Appl. Sci. J,* Vol. 31, No. 5, pp. 745-750.
10. Gillis S and Smith K A (1977), "Long Term Culture of Tumor-specific Cytotoxic T-cell Lines," *Nature,* Vol. 268, pp. 154 – 156.
11. Gillis S, Baker P E, Ruscetti F W and Smith K A (1978), "Long Term Culture of Human Antigen-Specific Cytotoxic T-cell Lines," *J. Exp. Med,* Vol. 148, No. 10, DOI: 10.1084/jem.148.4.1093.
12. Guyton A C and Hall J E (1998), *Textbook of medical physiology*, 6th Ed. Mcwiley publishers, London.
13. Hardy K and Chaudhri G (1997), "Activation and Signal Transduction Via Mitogen-activated Protein (MAP) Kinases in T lymphocytes," *Immunol. Cell Biol,* Vol. 75, No. 6, pp. 528-545.
14. Ijeh I I, Njoku O U and Ekenze E C (2004), "Medicinal Evaluation of *Xylopia aethiopia* and *Ocimum Gratissimum", J. Med. Aromatic Plant Sci*, Vol. 26, No. 1, pp. 19-21.
15. Ikeme M M and Adelaja A O (1990), "Effect

of the Timing Antigen Stimulation on Parasitaemia Profile and Subsequent Immunodepressing in an Experimentally Induced *Trypaosoma bruci* Infection," *Revue Elev. Med. Vét. Pays Trop,* Vol. 43, No. 3, pp. 331-336.

16. Ilori M O, Sheteolu A O,Omonigbehin E A and Adeneye A A (1996), "Antidiarrhoeal Activities of *Ocimum gratissimum* (Lamiaceae)", *J. Diarrh. Dis Res*, Vol. 14, pp. 283-6.

17. Kawasaki H, Onuki R, Suyama E and Taira K (2002), "Identification of Genes that Function in the TNFá- mediated Apoptotic Pathway Using Randomized Hybrid Ribozyme Libraries," *Nat. Biotechnol,* Vol. 20, No. 4, pp. 376-380.

18. Kevin MS, Burns JJ, Douglas ND, Nasha W and Steve O, *et al.* (2006), "Modulation of Cytokine Expression by Traditional Medicines: A Review of Herbal Immunomodulators", *Altern. Med. Rev*, Vol. 11, No. 2, pp. 128-150.

19. Krishnaraju A V, Rao C B M, Sundararaju D, Sengupta K and Trimurtulu G (2009), "Anti-Inflammatory Activity of *Vitex leucoxylon* L. Bark Extracts Against Freund's Complete Adjuvant Induced Arthritis in Sprague Dawley Rat," *Am. J. Infect. Dis*, Vol. 5, pp. 68-73.

20. Lam H Y, Yeap S K, Alitheen N B, Ho W Y and Omar A R, *et al*. (2010), "Understand the Role of Natural Killer", *Am. J. Immunol*, Vol. 6, pp. 54-61.

21. Lindequist U, Niedermeyer T H J and Julich W D (2005), "The Pharmacological Potential of Mushrooms", *Evidence-based Compl. Altern. Med,* Vol. 2, pp. 285–299.

22. Mattila P, Konko K, Eurola M, Pihlawa JM and Astola J, *et al.* (2001), "Contents of Vitamins, Mineral Elements, and Some Phenolic Compounds in Cultivated Mushrooms", *J. Agric Food Chem,* Vol. 49, pp. 2343-2348.

23. Mau J L, Chang C N, Huang S J and Chen C C (2004), "Antioxidant Properties of Methanolic Extracts from *Grifola frondosa, Morchella esculenta* and *Termitomyces albuminosus* Mycelia," *Food Chem,* Vol. 87, pp. 111–118.

24. Mayer G (2006), Immunology. Microbiology and Immunology On-line Textbook. Chapter One: Innate (non-specific) Immunity. USC School of Medicine. 2006. Htpp://pathmicro.med.sc.edu/ghafferar/innate.htm. Accessed on June 10, 2014.

25. Mitra S K, Gupta M and Sarma D N K (1999), "Immunomodulatory Effect of IM-133", *Phytother. Res*, Vol. 13, No. 4, pp.3 41-343.

26. Mizuno T (1995), "Bioactive Biomolecules and Mushrooms. Food Function and Medicinal Effects of Mushroom Fungi", *Foods Rev. Int*, Vol.11, pp.7–21.

27. Ngoupayo J, Tabopda T K and Ali M S (2009), "Antimicrobial and Immunomodulatory Properties of Prenylated Xanthones from Twigs of *Garcinia steudti", Bioorg. Med. Chem,* Vol. 17, pp. 5688-5695.

28. O'Sullivan R L, Lipper G and Lerner E A (1998), "The Neuroimmuno- cutaneous-endocrine Network: Relationship of Mind and Skin", *Arch. Dermatol,* Vol.134, pp.1431-143.

29. Obaseiki-Ebor E E, Odukoya K, Telikepalli

H, Mitscher L A and Shankel D M (1993), "Antimutagenic Activity of Extracts of Leaves of Four Common Edible Vegetable Plants in Nigeria (West Africa)", *Mutat. Res*, Vol.2, pp. 109-17.

30. Oladunmoye M K (2006), "Immunostimulatory Activity of Ethanolic Leaf Extract from *Ocimum gratissimum* in Albino Rat Orogastrically Dosed with *Escherichia coli* (NCIB 86)", *J. Pharmacol. Toxicol*, Vol. 1, pp. 389-94.

31. Oppenheim J J (2001), "Cytokines: Past, Present, and Future", *Int. J. Hematol*, Vol. 74, pp. 3-8.

32. Orafidiya L O, Fakoya F A, Agbani E O and Iwalewa E O (2005), "Vascular Permeability-Increasing Effect of the Leaf Essential Oil of *Ocimum gratissimum* Linn as a Mechanism for its Wound Healing Property," *Afr. J. Trad. Compl. Altern. Med,* Vol.2, pp.253-8.

33. Orafidiya L O, Adesina S K, Igbeneghu O A, Akinkunmi E O and Adetogun G E*, et al.* (2006), "The Effect of Honey and Surfactant Type on the Antibacterial Properties of the Leaf Essential Oil of *Ocimum gratissimum* Linn. Against Common Wound-Infecting Organisms", *Int. J Aromatherapy,* Vol. 16 pp. 57-62.

34. Pessoa L M, Morais S M, Bevilaqua C M L and Luciano J H S (2002), "Anthelmintic Activity of Essential Oil of *Ocimum gratissimum* Linn. and Eugenol Against *Haemonchus contortus," Vet. Parasitol,* Vol. 109, pp. 59-63.

35. Prabhu K S, Lobo R, Shirwaikar AA and Shirwaikar A (2009), "*Ocimum gratissimum*: a Review of its Chemical, Pharmacological and Ethnomedicinal Properties," *The Open Compl. Med, J,* Vol. 1, pp. 1-15.

36. Sahouo G B, Tonzibo Z F, Boti B, Chopard C and Mahy J P, *et al*. (2003), "Anti-inflammatory and Analgesic Activities: Chemical Constituents of Essential Oils of *Ocimum gratissimum*, *Eucalyptus citriodora* and *Cymbopogon giganteus* Inhibited Lipoxygenase L-1 and Cyclooxygenase of PGHS," *Bull. Chem. Soc. Ethiopia*, Vol.17, pp.191-7.

37. Schneider C R, Sheidt K and Brietmaier E (2003), "Four New Pregnantglycosides from *Gongronema latifolium* (Asclepidaceous)," *J. Parkische Chem. Chenisker-Zutung,* Vol. 353, pp.532-536.

38. Sharma S K, Atri R, Joshi, Ashu G and Arvind G (2012), "Evaluation of Wild Edible Mushrooms for Amino Acid Composition," *Acad. J. Plant Sci*, Vol.5, No.2, pp.56-59.

39. Sherman A R and Halliquest N A In: Brown M I (Ed.), *Present Knowledge in Nutrition*, 6th Ed. ILSI press. Washington DC.1990, Pp. 473-476.

40. Shin H J, Gye M H, Chung K H and Yoo B S (2002), "Activity of Protein Kinase C Modulates the Apoptosis Induced by Polychlorinated Biphenyls in Human Leukemic HL-60 Cells," *Toxicol. Lett*, Vol.135, No. 25–31.

41. Shinde U A, Phadke A S, Nair A A, Mungantiwar V S and Dikshit V S *et al.* (1999), "Preliminary Studies on the Immunomodulatory Activity of *Cedrusdeodara* Wood Oil," *Fitoterapia,* Vol. 79, pp. 333-339.

42. Smith K A (1998), "Interleukin-2: Inception, Impact and Implications," *Science*, Vol. 240, pp. 1169- 1176.

43. Sommer C (1999), "Animal Studies on Neuropathic Pain: The Role of Cytokines and Cytokine Receptors in Pathogenesis and Therapy," Schmerz, Vol.13, No.5, pp.315-323.

44. Sy G Y, Nongomierma R B, Sarr M, Cisse A and Fage B (2004), "Antidiabetic Activity of the Leaves of *Vernonia colorata* (Wild) Drake (*Composes*) in Alloxan Induced Diabetic Rats", *Dakar Medica*, Vol. 49, pp.36-39.

45. Tsai K D, Lin B R, Perng D S, Wei JC and Yu Y W, *et al.* (2011), "Immunomodulatory Effects of Aqueous Extract of *Ocimum basilicum* (Linn.) and some of its Constituents on Human Immune Cells", *J. Med. Plants Res,* Vol. 5, No. 10, pp. 1873-1883.

46. Wasser S P (2002), "Medicinal Mushrooms as a source of Antitumor and Immuno-modulating Polysaccharides," *Appl. Microbiol. Biotechnol,* Vol. 60, pp. 258–274.

47. Zhao Z, Jin J, Ruan J, Zhu C and Lim C, *et al.* (2007), "Antioxidant Flavonoid Glycosides from Aerial Parts of the Fern *Abacopteris enangiana," J. Nat. Prod,* Vol. 70, pp. 1683-1686.

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