Antiulcer and Blood-Boosting Activities of Feeds Supplemented with *Trametes versicolor* from Nigeria

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**Abstract**

Antiulcer and blood-boosting activities of feeds supplemented with *Trametes versicolor* (*Tv*) in Wistar rats (*Rattus norvegicus*) were studied. Haematological studies and antiulcer biochemical analysis were carried out on the rats using standard methods. Data were presented as Mean ± SEM, analyzed using two-way ANOVA and p ≤ 0.05 was significantly different in all the variables. Haematological parameters were not significantly different across all *Tv* treatments when compared with the control (CN). There were also significant differences in values obtained for gastric ulcer inhibition, nitric oxide, mucin, sulfhydryl, and H⁺/K⁺-ATPase. *Tv* treatment groups also differed when compared with ulcerated untreated control (CU). The pathological changes detected from histological studies on the stomach tissues showed inhibition of ulcers. *Tv* treatment groups demonstrated blood boosting and antiulcer activity through synergistic activities of mucin, H⁺/K⁺ ATPase activity, and antioxidant mechanism. The implications of these observations are discussed.

**Keywords:** *Trametes versicolor*, Antioxidant, Haematology, and Ulcer inhibition.

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**INTRODUCTION**

Ascomycetes and Basidiomycetes are major classes of higher fungi which form reproductive structures known as fruit bodies or basidiocarps [1]. Many higher fungi have been used in different countries of the world as sources of protein, dietary fibre, unsaturated lipids and different mineral elements [2-4]. Nigerian higher fungi have been reported to possess different medicinal properties [5-11]. Therapeutic values of fungi are directly linked to their possession of phytochemicals and bioactive compounds [12, 13]. These bioactive molecules could be extracted from edible, inedible and poisonous fungal species and characterized. In developing countries especially Nigeria, the medicinal uses and quest for new health and nutritional supplements from fungi have been exploited by scientists and indigenous people. Medicinal fungi have been used in the management of several diseases [14-16]. Problems related to the use of standard drugs are being addressed by the advocacy for the exploitation and use of herbal products as alternatives.

Different fungi have been used from ancient times, by traditional healers from Asia, Africa, America and Europe. Traditional medical practitioners in South-Western Nigeria usually prepare hot water extracts of fungi with other medicinal plants, or they may use local gin for the extraction of these fungi [5, 8].

*Trametes versicolor* is a polypore fungus. It is also known as *Polyporus versicolor* or *Coriolus versicolor*. This fungus has been reported as possessing antioxidant, immunomodulatory and anti-inflammatory [17, 18], anticancer [19], antimicrobial [11], and prebiotic [19] properties.

Blood is the transport medium in the body of all mammals. Anything that affects blood, usually affects the entire body in relation to growth, health, body maintenance, and reproduction. However, nutritional factors usually influence blood status of any animal [20, 21]. Blood parameter assessment can be used to determine the effects of foreign materials such as medicinal herbs.
Gastric Ulcer (GU) is known as an ulcer of the stomach and is defined as sub-mucosal or deeper erosion of normal gastric mucosa. Peptic Ulcer Disease (PUD) is predominantly induced by *Helicobacter pylori*. Non-steroidal anti-inflammatory medicines have been linked to the increased secretion of gastric acids. Anxiety, smoking, spicy foods and dietary deficiencies are the other factors. Peptic ulcer disease risks may include internal bleeding, gastro-intestinal blockage, perforation, and peptic ulcer refractory [21, 22]. Peptic ulcer disease is significant worldwide, affecting 4% of the total population. There were 327,000 deaths from PUD in 1990 and 301,000 deaths in 2013 [22]. Every year, 4 million people throughout the world are affected by PUD. Accordingly, conventional medicine manages ulcers with proton pump inhibitors (PPIs), H2 receptor antagonists, antacids, antibiotics, and mucosal protection agents [23]. Nevertheless, there are reports of negative effects and long-term recurrences from these medications. Consequently, people are exploring other alternatives, including natural remedies.

Macrofungi from Nigeria have been reported of possessing secondary metabolites, which has made them to be reservoirs of useful bioactive compounds with valuable therapeutic values [1, 12, 16]. However, there is a dearth of information on the haematonic or antulcer properties of *Trametes versicolor*. The purpose of this study was to examine the antiulcer and blood boosting activities of *Trametes versicolor* from Ibadan, southwestern, Nigeria.

**MATERIALS AND METHODS**

Fresh fruit bodies of wild *Trametes versicolor* were collected during the rainy season (August-September, 2017) from University of Ibadan Botanical Gardens, Ibadan (7.3775° N, 3.9470° E) in Oyo State, South-West Nigeria. This preliminary identity was validated and further subjected to the standard descriptions of Ostry et al. [24]. The fungal samples were air-dried, powdered and preserved for future use in an air-tight amber bottle, and refrigerated at 4°C.

![Fig-1: A photograph of *Trametes versicolor*](image)

**Rat Experiments**

Thirty five Wistar rats (100-110g; n=7) were divided into five groups thus: Groups 1- (un-ulcerated normal feed (CN)); 2- (ulcerated not treated (CU)); 3- (20 mg/kg of cimetidine (Cm)); 4- (20% of Tv) and 5- (40% of Tv) for days 7 and 14 respectively. Full haematological analysis was carried out on the rats at the end of each experimental day. The animals had free access to their normal feeds for the control experiments and were pretreated with *Tv* and cimetidine for 7 and 14 days respectively with water ad *libitum* throughout the experiment. They were then fasted for 24 hrs prior to the administration of indomethacin and sacrificed after 4 hours. The stomach of each rat was excised, weighed, and graded for ulceration. Animal experiments were performed in line with Experimental Animal Care and Use Guidelines of the National Institute of Health (Pub No. 85-23, revised 1985). Biochemical and histological studies were carried out on the excised stomach tissues using standard methods.

**Determination of Full Blood Cell Count**

The Dacie and Lewis [25] methods were used for haematological research in the analysis of blood.

**Macroscopic Assessment Scoring of Ulcer**

Inas et al. [26] method was used for indomethacin-induced ulceration while gastric ulceration was assessed using Elegbe and Bamigbose [27] established scoring technique.

**Histopathological Studies**

For the histological studies, the method Elegbe and Bamigbose [27] was used. Biochemical determination of Lipid peroxidation, Nitric oxide (NO), Sulphhydryl content, Mucosal Hydrogen-peroxide (H2O2), Total protein concentration, Hydrogen/Potassium anti-pump activities, and Mucoin content.

The lipid peroxidation was calculated by the method of Varshney and Kale [28], and the concentration of nitrite in the supernatant was measured as an NO production indicator detected by the Griess reaction [17]. Sulphydryl levels and the Hydrogen peroxide (H2O2) tissue activity was carried out using the principles of Elegbe and Bamigbose [27]. The protein concentrations of the various samples were estimated using the Biiaret process, as defined by Elegbe and Bamigbose [27], with a slight modification; and the determination of Hydrogen/Potassium anti-pump activities was carried out using the method Ronner et al. [29], as updated by Bewaji et al. [30]. Determination of mucin content was carried out by using the method of Bewaji et al. [30].
STATISTICAL ANALYSIS

Data were expressed as Mean ± SEM, analyzed using two-way ANOVA and p ≤ 0.05 was significant.

RESULTS

Table 1: Influence of Trametes versicolor supplemented diets on packed cell volume, erythrocyte sedimentary rate, reticulocyte, haemoglobin, and red blood cell and platelets counts

<table>
<thead>
<tr>
<th>Groups</th>
<th>PCV (%)</th>
<th>ESR (mm/hr)</th>
<th>RECTIC (x 10^3/L)</th>
<th>HB (g/dL)</th>
<th>RBC (x10^12/L)</th>
<th>PLATELETS (x10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN</td>
<td>41±0.5</td>
<td>49.3±2.3</td>
<td>1.13±0.7</td>
<td>2.4±0.2</td>
<td>3.3±0.4</td>
<td>7.2±4.0</td>
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<tr>
<td></td>
<td>Day 7</td>
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<tr>
<td>20Tv</td>
<td>44±1.5</td>
<td>51.3±2.7</td>
<td>1.27±0.8</td>
<td>14.6±0.7</td>
<td>16.9±0.6</td>
<td>7.4±0.3</td>
</tr>
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<tr>
<td>40Tv</td>
<td>43±0.7</td>
<td>50±0.0</td>
<td>2.5±0.6</td>
<td>14.8±0.8</td>
<td>16.4±0.6</td>
<td>7.4±0.0</td>
</tr>
<tr>
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<td>Day 7</td>
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<td>7.2±4.0</td>
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<td>Day 14</td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 14</td>
</tr>
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<td>51.3±2.7</td>
<td>1.27±0.8</td>
<td>14.6±0.7</td>
<td>16.9±0.6</td>
<td>7.4±0.3</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 14</td>
</tr>
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<td>50±0.0</td>
<td>2.5±0.6</td>
<td>14.8±0.8</td>
<td>16.4±0.6</td>
<td>7.4±0.0</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 14</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM. (n = 3). All values were not significantly different from control normal (CN) at p<0.05. 20Tv= 20% w/w of Trametes versicolor in feed, 40Tv= 40%w/wof Trametes versicolor in feed, Control normal (CN), -control group not ulcer-induced.

Influence of Trametes versicolor supplemented diets on packed cell volume, erythrocyte sedimentary rate, reticulocyte, haemoglobin, red blood cell, and platelet counts on Wistar rat is shown in Table 1. On both days of treatments, no significant differences in the above haematological variables were observed with 40Tv and 20Tv when compared with CN. Haematological analysis was performed on the effect of Trametes versicolor supplemented diets on Wistar rats, and their results showed no significant effects.

Table 2: Influence of Trametes versicolor supplemented diets on white blood cell, lymphocyte, and neutrophils, monocyte, and eosinophils counts in Wistar rat

<table>
<thead>
<tr>
<th>Group</th>
<th>WHITE BLOOD CELL(10^3/μL)</th>
<th>LYMPHOCYTES (10^3/μL)</th>
<th>NEUTROPHILS (10^3/μL)</th>
<th>MONOCYTE (10^3/μL)</th>
<th>EOSINOPHILS (10^3/μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN</td>
<td>4183±44</td>
<td>5733±20</td>
<td>71.3±1.8</td>
<td>13.7±0.3</td>
<td>1.3±0.33</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 7</td>
<td>Day 7</td>
<td>Day 7</td>
</tr>
<tr>
<td>20Tv</td>
<td>6483±44</td>
<td>3516.7±30</td>
<td>72.7±4.3</td>
<td>15.7±0.5</td>
<td>1±0</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 7</td>
<td>Day 7</td>
<td>Day 7</td>
</tr>
<tr>
<td>40Tv</td>
<td>5067±33</td>
<td>3416.7±25</td>
<td>68.3±5.7</td>
<td>17±0.5</td>
<td>1.3±0.33</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 7</td>
<td>Day 7</td>
<td>Day 7</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM. (n = 3). All values were not significantly different from control normal (CN) at p<0.05. 20Tv= 20% w/w of Trametes versicolor in feed, 40Tv= 40%w/wof Trametes versicolor in feed, Control normal (CN), -control group not ulcer-induced

Influence of Trametes versicolor supplemented diets on white blood cells (WBC), lymphocytes, neutrophils, monocytes, and eosinophils counts are shown in Table 2. No observable differences in the haematological variables were noticed with 40Tv and 20Tv with CN on both days of treatments.

Table 3: Influence of Trametes versicolor supplemented diets on Albumin, Globulin, Blood urea nitrogen, and Creatinine on rat

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALBUMIN (g/dL)</th>
<th>GLOBULIN (g/dL)</th>
<th>BUN (g/dL)</th>
<th>CREATININE (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN</td>
<td>2.8±0.3</td>
<td>2.9±0.18</td>
<td>4.5±0.2</td>
<td>17.27±0.35</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 7</td>
<td>Day 7</td>
</tr>
<tr>
<td>20Tv</td>
<td>2.9±0.25</td>
<td>3.6±0.12</td>
<td>4.67±0.08</td>
<td>17.7±0.15</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 14</td>
<td>Day 14</td>
</tr>
<tr>
<td>40Tv</td>
<td>3.1±0.06</td>
<td>3.27±0.3</td>
<td>4.53±0.07</td>
<td>17.53±0.09</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 7</td>
<td>Day 7</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM. (n = 3). All values were not significantly different from control normal (CN) at p<0.05. 20Tv= 20% w/w of Trametes versicolor in feed, 40Tv= 40%w/wof Trametes versicolor in feed, Control normal (CN), -control group not ulcer-induced

Influence of Trametes versicolor supplemented diets on Albumin, Globulin, Blood urea nitrogen, and Creatinine on the rats is shown in Table 3. It was observed that on both days of treatments, there were no significant effects in the above serum biochemical variables with 20Tv and 40Tv when compared with CN.
Table 4: Influence of *Trametes versicolor* supplemented diets on ulcer score, ulcer index and ulcer percentage inhibition of Indomethacin-Induced Gastric Ulcerated rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ulcer score (mean ± SEM)</th>
<th>Ulcer index (mm²)</th>
<th>Percentage inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 7</td>
</tr>
<tr>
<td>CN</td>
<td>0.0±0</td>
<td>0.0±0</td>
<td>0.0</td>
</tr>
<tr>
<td>CU</td>
<td>6.0±2.3</td>
<td>6.0±2.3</td>
<td>0.18</td>
</tr>
<tr>
<td>Cm</td>
<td>3.5±1.2</td>
<td>1.83±0.4</td>
<td>0.11</td>
</tr>
<tr>
<td>20Tv</td>
<td>3.5±1.2</td>
<td>2.17±0.5</td>
<td>0.11</td>
</tr>
<tr>
<td>40Tv</td>
<td>5.67±1.6</td>
<td>3.67±1.03</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Values were expressed as Mean ± SEM. (n = 3). 20Tv = 20%w/w of *Trametes versicolor* in feed, 40Tv = 40%w/w of *Trametes versicolor* in feed. Control normal (CN)-control group not ulcer-induced. Ulcer untreated control (CU). (Cm)-Control treated with 20 mg/kg of cimetidine.

Influence of *Trametes versicolor* supplemented diets on ulcer score, ulcer index, and ulcer percentage inhibition of Indomethacin-Induced Gastric Ulcerated is presented in Table 4.

It was observed that both treatments of 20%w/w *Trametes versicolor* (20Tv) and 40%w/w *Trametes versicolor* (40Tv) significantly increased in ulcer percentage inhibition by day 7 and day 14 when compared to CU. However, the highest inhibition of ulcer percentage was observed after day 14 for both 20Tv and 40Tv.

Plate 1: Photomicrograph of stomach section Day 7 stained by Haematoxylin and Eosin stain MAG. X100 showing; 7CN: There is no observable lesion in the gastric mucosa, 7CU: There is marked ulceration, necrosis of chief cells and inflammation in the gastric mucosa (U), 7Cm: There is erosion of the gastric mucosa (E), 7 Tv 20: There is necrosis and loss of gastric mucous cells, 7 Tv 40: There is no observable lesion.

Plate 2: Photomicrograph of stomach section (Day 14 stained by Haematoxylin and Eosin stain MAG. X 100) showing; 14CU: There is ulceration of the mucosa with hemorrhagic exudate (U), 14CN: There is no observable lesion, 14Cm: There is loss of surface mucous cells (SMc), 14Tv 20: There is no observable lesion, 14Tv 40: There is no observable lesion.
Figure 2 shows the influence of 20Tv and 40Tv diets on the total protein content of Indomethacin-Induced Gastric Ulcerated rats for exposure periods of 7 and 14 days. On days 7 and 14 of treatment, no significant increases ($p < 0.05$) were observed with treatment groups formulated with 20Tv and 40Tv except 40Tv ($p < 0.001$) after day 14 of treatment as compared to CU.

No significant differences were observed on MDA with 20Tv and 40Tv on days 7 and 14. No significant decrease was found with 20Tv and 40Tv relative to CU for the contrast between days 7 and 14 treatments.

On day 7, only supplemented group of 20Tv increased significantly compared to CU. For day 14, the nitric oxide content of both 20Tv and 40Tv increased significantly as compared to CU; however, significant increase was only observed with 40Tv treatment when comparing 7 and 14 day treatments.
On day 7, a significant increase ($p < 0.001$) was only observed with 40Tv while a significant increase ($p < 0.001$) was observed with 20Tv on day 14 when compared with CU. Comparing day 7 and day 14, significant differences ($p < 0.001$) were observed with both 20Tv and 40Tv.

No significant increase was observed on days 7 and 14 in CU with 20Tv and 40Tv treatment groups. Similarly, no significant differences with treatment days were noticed.
On days 7 and 14, all the treatment groups except 40Tv on day 14 showed significant decrease in H+/K+ ATPase pump activity compared with CU.

Comparing day 7 and day 14 treatments, no significant difference (p< 0.05) was observed except with CN treatment groups.

![Fig-8: influence of Trametes versicolor supplemented diets on Mucin content of indomethacin induced ulcer in rats for 7 and 14 days exposure periods](image)

Keys of significance: Following one–way Anova, 'p' < 0.05, "p' < 0.01, ""p' < 0.001 at 7 days and 'p' < 0.05, "p' < 0.01, ""p' < 0.001 at 14 days compared with the corresponding ulcer untreated control (CU). Using two-way Anova, 'p' < 0.05, "p' < 0.01, ""p' < 0.001, between 7 and 14 days' exposure periods. 20Tv= 20% w/w of Trametes versicolor in feed, 40Tv = 40% w/w of Trametes versicolor in feed. Control normal (CN) -control group not ulcer-induced. Ulcer untreated control (CU). Control treated with 20 mg/kg of cimetidine (Cm).

On days 7 and 14, a significant increase ('p' < 0.001) in Mucin contents was observed with 20Tv and 40Tv; however, comparing days 7 and 14 treatments, both 20Tv and 40Tv showed significant increases ("p' < 0.001) in Mucin content on day 14.

DISCUSSION

From our results, Trametes versicolor supplemented diets do not affect erythropoiesis negatively. This observation is similar to the findings of Togun et al. [31], who reported that the increase in PCV in combination with the marginal increase in RBC shows more successful erythropoiesis in experimental rabbits. As observed in the results, increase in WBC observed on day 7 could be due to the adjustment of the rats to the formulated feed which was a foreign substance in their bodies while decrease observed in day 14 treatment period revealed the immunomodulatory property of the supplemented feeds.

There were no major differences in neutrophils, lymphocytes, and monocytes seen, thus further confirming the results. Analysis of the renal and hepatic function was highly useful in screening for toxicity of medicine and herbal extracts, as both are essential to an organism’s survival [32]. Trametes versicolor supplemented diets showed no sign of toxicity from the serum biochemical parameters indicating that the role of hepatocyte in rats will not be affected by sub-chronic feed intake. However, the existence of bioactive compounds known to have antioxidant and anti-inflammatory activities may be linked to their antiulcer property as observed with the percentage inhibition of the Tv supplemented diets [17]. This antiulcer property was further observed with the histological studies which showed healing features, such as the absence of observable lesions with the Tv treatments. The increase in total protein content observed may be due to the presence of high protein contents in mushrooms which is mostly needed during inflammation for cell regeneration and repair. This is in support of Jonathan et al. [33] findings, which reported that macro-fungi are highly rich in protein content.

The test of oxidative activities that used malondialdehyde assay as its marker showed that there was no breakdown of the lipid stores on the cell membrane due to the treatments. It also discloses the capability of the treatments to avoid an increase in the generation of free radicals that might have aggravated the induced gastric ulcer. The increase in NO as observed may have conferred antiulcer properties on the rats. Nitric oxide (NO) helps protect the integrity of the mucus membrane and stomach epithelium which helps to mediate gastric blood flow as a vasodilator and prevent the secretion of acids as well as stimulate the production of mucus and bicarbonate. It thereby gave protection to the gastrointestinal tract which is useful in gastric ulcer healing [34]. The increase in sulphydryl level suggests gastro-protective effects of Trametes versicolor supplemented diets which is useful for the formation and maintenance of gastric mucus through the growth of disulfide bridges which limits the development of reactive oxygen species associated with tissue injury while maintaining gastric integrity [35].

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Hydrogen peroxide assay was further used to assess Trametes versicolor's antioxidant properties in the feed. The observed increase may be due to the presence of bioactive agents with antioxidant properties which helped to confer antiulcer properties on the fungus. This is in agreement with the findings of the Oyedemi et al. [36], that artificial and biological antioxidants were required to avoid negative effects of unpaired radicals.

The changes seen with the higher fungi supplemented feeds on Hydrogen/Potassium pump activities in the experimental rats could be attributed to antiulcer activity demonstrated by the test fungus. Trametes versicolor could have acted as gastric proton pump inhibitor. This is in agreement with the findings of Strand et al. [37] who reported that the primary goal of doctors when managing peptic ulcer is treating with drugs that promote proton pump inhibitor in order to reduce gastric acid secretion. The increase in mucin content may have conferred potential antiulcer properties on the Trametes versicolor supplemented diets by helping to maintain homeostasis through its mucosal defense system [38].

From the results obtained, it can be concluded that Trametes versicolor treatment groups demonstrated blood boosting and antiulcer activity at both concentrations through the synergistic activities of mucin, H+K+ ATPase activity, and antioxidant mechanism.

The results of this study suggest that diets enriched with Trametes versicolor do not contain the toxic effects that might hinder their therapeutic use as herbal medicine for the treatment of blood deficiencies and ulcer.

REFERENCES