EFFECT OF GARNODERMA RESINACEUM EXTRACTS ON THE HAEMATOLOGICAL AND HISTOLOGICAL PROPERTIES OF SWISS ALBINO RATS INFECTED WITH BACILLUS CEREUS AND ESCHERICHIA COLI.

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ABSTRACT
Studies were carried out on the effect of methanollic extract of *Ganoderma recinaceum* (mushroom) haematology and physiopathology of Swiss albino rats infected with *Bacillus cereus* (BCC) and *Escherichia coli* (ECD). The results showed that, packed cell volume (PVC) for the uninfected animal (CON), was 48.67± 6.50. Those infected with BCC and ECD plus *Ganoderma recinaceum* treatment gave 45.00± 6.56 and 46.37± 14.43 PVC respectively. However, the blood cell count for the control (CON) was 4.93± 1.02 while infected rats treated with *Ganoderma recinaceum* gave 5.63± 2.36 and 5.13± 0.49 for BCC and ECD infections respectively. Histological examination showed necrosis of heart muscles, haemorrhage and thickened hepatocytes of the liver. These degenerating conditions posed by *Bacillus cereus* were greatly reduced after treatment with the mushroom extract. Also the reduced glomerula of the kidney and elongation of the microvilli of the small intestine were noticeable reduce on treatment with the mushroom extract. This implies that the extract may possess properties that can be used as anti-necrotizing agent. *Ganoderma recinaceum* extract can therefore be used in the treatment of *B. cereus* infections.

Key Words: Physiopathology, Heamatology, *Ganoderma recinaceu*, Swiss Albino rats.

INTRODUCTION
Mushrooms are major sources of potent pharmaceutical products and over 300 species of mushrooms have been identified to possess medicinal properties (Klaus & Miomir, 2007). Several externalized secondary metabolites and extracellular secretions by mushrooms are able to combat diseases from bacteria and protozoa such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Plasmodium falciparum* by producing antibiotics that prevent the growth and multiplication of these organisms (Hobbs, 2006). *Ganoderma* species were first indexed as longevity, health promoting and tonic herb of the superior class, and has been used in Traditional Chinese Medicine (TCM) for more than 2000 years to prevent and treat various human diseases, tumour growth and immunological disorder (Xiao, et al., 2007). Fruiting bodies of *Ganoderma* species occur in different morphological types with characteristic biological properties. These
Mushrooms have been regarded as a panacea for many types of diseases and debility due to prolonged illness by providing a protective immunological shield against various infections (Lin, 2001). *Ganoderma* species are capable of strengthening body resistance and improving constitutive homeostasis in patients. This present study investigates the effect of *G. resinaceum* extract on Swiss albino rats infected with *Bacillus cereus* and *E. coli*.

**MATERIALS AND METHODS**

**Collection and extraction of Mushroom Sample**

Fruiting bodies of *Ganoderma resinaceum* were collected from Igunshin forests in Akure South Local Government Area of Ondo State, Nigeria. Samples were identified and authenticated in the Department Botany and Microbiology, University of Ibadan. The mushroom was cleaned of foreign particles with a soft brush, washed with clean water and dried in an oven at 45 °C. The dried mushrooms were milled into powder using blender and 20g of it was extracted using 200ml of 60% methanol for 72 hours. The mixture was filtered through a Whatman No.1 filter paper and the filtrate concentrated in a rotary evaporator.

**Experimental Animals**

Male and female Swiss albino rats (8 - 10 weeks old) weighing 100–120g were obtained from the Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria. The rats were housed in metal cages with constant temperature of 25-30°C, 12/12h light/dark cycle and maintained on standard rat diet with free access to clean water *ad libitum*. The experimental animals were allocated into two groups (control and tested group). One thousand milligram per millimetre per kilogram body weight of *G. resinaceum* extract was administered orally to the test group while the control group received sterile distilled water. Thereafter the animals were observed for various signs and symptoms such as hyperactivity, sluggishness, anorexia and diarrhoea for 21 days. The animals were sacrificed according to the Institutional committee for ethics in animal experiment and the blood collected in EDTA bottles for haematological analyses while organs namely the heart, liver, kidney and small intestine were removed, washed in normal saline, blot dry with filter paper, weighed and then fixed in 10% formalin for histological study.

**Statistical Analysis of Data**

The data obtained during the investigations were subjected to Analysis of Variance and inferences made at P<0.05 using the SPSS 15.0 software package. Duncan’s New Multiple Range Test was used to separate means.
Table 1: Haematological analysis of the Blood of Infected Rats

<table>
<thead>
<tr>
<th></th>
<th>PCV%</th>
<th>WBC/mm$^3$ ($\times 10^3$)</th>
<th>LPT%</th>
<th>NTP%</th>
<th>MNT%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>48.67 ± 6.5</td>
<td>4.93 ± 1.02</td>
<td>36.67 ± 3.06</td>
<td>59.33 ± 8.96</td>
<td>0.33 ± 0.58</td>
</tr>
<tr>
<td>BCA</td>
<td>40.67 ± 9.71</td>
<td>4.10 ± 3.34</td>
<td>49.33 ± 10.60</td>
<td>49.33 ± 9.71</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>ECB</td>
<td>49.67 ± 2.52</td>
<td>5.57 ± 1.15</td>
<td>49.33 ± 6.50</td>
<td>50.33 ± 7.02</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>BCC</td>
<td>45.00 ± 6.56</td>
<td>5.63 ± 2.36</td>
<td>74.66 ± 11.15*</td>
<td>25.00 ± 10.58*</td>
<td>0.33 ± 0.58</td>
</tr>
<tr>
<td>ECD</td>
<td>46.37 ± 14.43</td>
<td>5.13 ± 0.49</td>
<td>51.33 ± 12.50</td>
<td>48.67 ± 12.50</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>GTE</td>
<td>54.33 ± 7.64</td>
<td>4.29 ± 1.29</td>
<td>55.33 ± 11.72</td>
<td>44.33 ± 11.93</td>
<td>0.33 ± 0.58</td>
</tr>
</tbody>
</table>

Mark “*” showed significant difference, Values are mean ± SD (n=3).

CON= Control group of the experiment, BCA=Rats infected with *Bacillus cereus* without treatment, ECB= Rats infected with *E. coli* without treatment, BCC= Rats infected with *Bacillus cereus* with treatment, ECD= Rats infected with *E. coli* infected with treatment, GTE= Methanol extracts only, PCV=Packed cell volume, WBC=White blood cell, LPT=Lymphocyte, NTP=Neutrophil, MNT=Monocytes.

Plate 1a. : Photomicrograph of liver section of uninfected rats. H+E x100
Plate 1b. : Photomicrograph of liver section of rats infected with *Bacillus cereus* without treatment showing haemorrhage (arrow). H+E x100
Plate 1c. : Photomicrograph of liver section of rats infected with *Bacillus cereus* and treated with GTE showing thickening of the hepatocytes. H+E x100
Plate 2a: Photomicrograph of kidney section of uninfected rats. H+E x 100
Plate 2b: Photomicrograph of kidney section of rats feed with GTE showing cellular infiltration and the detachment of the glomerula (arrows). H + E

Plate 2c: Photomicrograph of kidney section of rats infected with *Bacillus cereus* showing haemorrhage of the glomerula. H + E X 100

Plate 3a: Photomicrograph of small intestinal section of uninfected rats. H + E X 100

Plate 3b: Photomicrograph of small intestinal section of *Bacillus cereus* infected rats showing elongation of villi and slight erosion of the crypt of the intestinal glands. H + E X 100

Plate 3c: Photomicrograph of small intestinal section of rats fed with GTE after infected with *Bacillus cereus* showing slight degeneration of the intestinal villi. H + E X 100

Plate 4a: Photomicrograph showing smooth muscles of the heart for uninfected rats. H + E X 100

Plate 4b: Photomicrograph of smooth muscles of the heart infected with *Bacillus cereus* showing necrosis (arrow). H + E X 100

Plate 4c: Photomicrograph of the heart muscles showing expansion and slight degeneration (arrows) after treatment. H + E X 100
package. Duncan’s New Multiple Range Test was used to separate means.

**RESULTS AND DISCUSSION**

Table 1 shows the results of haematological analyses of uninfected and infected rats with decrease in PCV of BCA, BCC and ECD while an increase in WBC of ECB, BCC and ECD was recorded. This is an indication of damages to the erythrocyte membrane and hemolytic anaemia of the rats. *Bacillus cereus*, produces toxin in the small intestine causing diarrhea and sometimes vomiting (Burke, 2009). Results revealed haemorrhage and thickened hepatocytes however the extract was able to prevent haemorrhage in the liver section of rats after treatment with GTE (Plate 1a – 1c), therefore GTE can serve as a potential source of hepatoprotective agents against *B. cereus* infections. The liver is a vital organ of human body which performs detoxification of the exogenous drugs, viral infections and chronic alcoholism. During these processes, the liver goes under stress resulting in liver diseases, liver failure, serious health problems and possibly death (Wolf, 1999). Therefore, ability of GTE to preserve the normal hepatic physiological mechanisms is an index of its protective effect.

There was haemorrhage, infiltration of cells, reduction and detachment of the glomerula of the kidney section (Plate 2a – 2c) while sections of the small intestine revealed elongation, degeneration, cellular infiltration and the congestion of microvilli (Plate 3a – 3c). Composition of food changes as it passes down the intestinal tract due to variations in the rate and type of absorption may cause an equivalent change in intestinal structure.

The smooth muscles of the heart (Plate 4a – 4c) showed necrosis, expansion and wavy contraction band of the sections. The heart is the site where blood is circulated to other parts of the body for various processes and the kidney is the site for filtration and reabsorption. There was no pathological effect of the heart muscles of rats infected with *E. coli* and those treated with the mushroom extract confirming the fact that *E. coli* do not colonise the heart. The degenerating condition posed by *Bacillus cereus* was reduced after treatment with the mushroom extract. This implies that the extract may possess properties that can be used as anti-necrotizing agent. It has also been reported that the safety assessment in experimental animals of both medicinal and non-medicinal biologically active chemicals has been very successful in predicting toxicity in humans (Zhao et al., 1995). Sasidharan et al., (2008) reported that toxic effects of natural products on host cells must be considered as a substance that may exhibit an apparent biological activity by virtue of toxic effect on cells. Ogboninia, et al., (2008) reported that experimental screening is important to ascertain the safety, efficacy and components of natural products. The ingestion of aqueous extract of *G. resinaceum* showed a promising pharmacological property.
REFERENCES


