BIOPROTECTIVE POTENTIAL OF BACTERIOCINS FROM SOME LACTOBACCILLUS SPECIES ISOLATED FROM FOODS

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ABSTRACT
Lactobacillus species isolated from ogi, kunnu, yoghurt and palm wine were found to produce bacteriocins. The bacteriocins had broad spectra of antimicrobial activities against both Gram-positive and negative bacteria. The effects of the bacteriocins on Escherichia coli infections in rats were evaluated. Sprague-Dawley rats were infected with E. coli and treated with 1280 AU/ml of the bacteriocins from L. plantarum MO21, L. plantarum MP12, L. casei MK21, L. casei MO11, L. brevis MK11 and L. buchneri MY21. Escherichia coli infection caused upregulation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total protein, globulin, cholesterol, bilirubin and glucose levels in sera of the infected rats which were down-regulated in the bacteriocin treated rats. Gastric and GIT damage caused by E. coli infection were reduced in the bacteriocin-treated groups. Therefore, it is concluded that these bacteriocins may have useful biomedical applications.

Keywords: Lactobacillus species; bacteriocin; gastric tissue; total protein and globulin

INTRODUCTION
The lactic acid bacteria (LAB) are rod-shaped bacilli or cocci characterized by an increased tolerance to a lower pH range. This aspect partially enables LAB to outcompete other bacteria in a natural fermentation, as they can withstand the increased acidity from organic acid production e.g. lactic acid. Lactic acid bacteria are generally recognized as safe (GRAS) and play an important role in food and feed fermentation processes. Lactic acid bacteria are generally considered to have probiotic attributes and are used in the production of fermentation products such as starter culture added under controlled conditions. The preservative effect exerted by lactic acid bacteria is mainly due to the production of organic acids, particularly lactic acid. Probiotics are live microorganisms that when consumed in an adequate amount and to prevent respiratory infection in children attending daycare centers. It is also used for skin disorders such as fever blisters, canker, sores, eczema (Woo et al., 2010), acne, high cholesterol, lactose intolerance and to boost the immune system (Berggren et al., 2011). Lactobacilli have been used for decades against infections in both healthy and immunocompromised individuals. Several studies have shown that these organisms have been used for the production of probiotics and other lactic acid bacteria to enhance the digestive system and improve health. Therefore, these organisms have been widely used as probiotics (Tannock, 1999). Many of these lactic acid bacteria are known to produce antibacterial substances such as bacteriocins which can inhibit the growth of several pathogenic bacteria. Lactobacillus is a genus of bacteria that are natural antimicrobial peptides or small proteins with bactericidal or bacteriostatic activity against genetically related species (Klaenhammer, 1988). Bacteriocin can be classified broadly as those synthesized by Gram-positive and those by Gram-negative organisms, among those synthesized by Gram-positive, Lactobacilli bacteriocin is of commercial value (Garneau et al., 2002).

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Probiotics are live microorganisms that when consumed in an adequate amount as part of the food, confer a health benefit on the host (FAO/WHO, 2001). An experimental focus of probiotic LAB strains has indicated that this potential might play a considerable role during in vivo interactions occurring in the human gastro intestinal tract (Avonts and De Vuyst, 2001; Kim et al., 2003). Therefore the aim of this research is to isolate and identify Lactobacillus species from some foods and to carry out in vivo assay of the bacteriocin produced by the isolated Lactobacillus species.

MATERIAL AND METHODS

Sample Collection
Four food samples which are palm wine, ogi, kunnu and yoghurt were purchased from local producers in Ogbomosho, Oyo State, Nigeria.

Microbiological Analysis
One millilitre of each sample was measured, dispensed into 9 mL of sterile peptone water in McCartney bottles, and homogenized by thorough shaking. The samples were diluted into 10-fold dilutions and appropriate dilution was plated on deMann Rogosa Sharpe agar (Oxoid) to obtain LAB. Plates were incubated at 30°C for 48 h under anaerobic conditions. Colonies were randomly picked from
the agar plates and the strains were streaked out repeatedly to check for purity and sub-cultured on fresh agar plates of the isolation media, followed by microscopic examinations. The stock cultures were routinely maintained on MRS agar slants kept in refrigerator at 4°C. The organisms were kept in freezing medium, by inoculating pure cultures into MRS broth with 20% glycerol and stored at −20°C.

Phenotypic Characterization

The cell morphology of the presumptive LAB strains were viewed by using a phase contrast microscope (Olympus CH3-BH-PC, Japan) after Gram staining and testing for catalase activity. Strains were preliminarily identified based on phenotypic properties such as the ammonia (NH₃) production from arginine, growth at 15°C and 45°C, ability to grow at pH 6.5% of sodium chloride (NaCl), hydrogen peroxide production, pH reduction in MRS broth, and gas (CO₂) production from glucose, according to Dykes (1995). All the strains were tested for the sugar fermentation patterns.

Identification of Strains

Strains identification was carried out according to physiological and biochemical characteristics, as described by Schleifer and Kllipper-Balz (1984). To confirm the identity of the isolates, total genomic DNA was extracted using the method described by Oladipo et al. (2013). Identification was carried out by sequencing of the 16S rRNA genes using the primers designated as FD1 (5’-AGAGTTTGATCCCTGCGTCAAG3’- forward) and RD1 (5’-AAGGAGGTGATCCAGCCGCG3’) for reverse (Weisburg et al., 1991).

Determination of bacteriocin production and antimicrobial spectra

This was carried out using the modified method of Oladipo et al. (2014a, b; 2015a). Briefly, Lactobacillus species were propagated in 100mL MRS broth for 72 hours at 30°C anaerobically. For extraction of bacteriocin, a cell-free solution was obtained by centrifuging the culture (6708 g for 20 minutes at 4°Cwith Beckman LS505B) and was adjusted to pH 7.0 by means of 1M NaOH to exclude the antimicrobial effect of organic acid. Inhibitory activity from hydrogen peroxide was eliminated by the addition of 5mg/mL catalase and filter sterilized through 0.22 mm filters. The antimicrobial activities of the supernatants were determined by well diffusion assay, 10µL aliquots of supernatant were placed in wells (3-mm diameter) cut in cooled soft LB agar plates previously seeded with indicator microorganisms. After 2 hours at 4°C, the plates were incubated at 37°C for three weeks. The control had the highest weight gained after the first, second and third week while the infected but not treated group showed the lowest weight gain.

Phenotypic characteristics of the isolates and the bacteriocin produced showed antimicrobial activity against Salmonella typhimurium, Enterococcus faecalis, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumonia, Enterobacter cloacae and Pseudomonas aeruginosa. The culture supernatant of Lactobacillus species was found to possess’ antimicrobial activity against Gram positive and Gram negative bacteria. This supports the previous findings of Nes et al. (1996) who reported that bacteriocins are proteinaceous compounds with inhibitory activity against more or less related bacterial genera. Juven et al. (1992) also reported that the ability of the Lactobacilli to produce metabolites such as bacteriocins has been suggested as being responsible for their ability to inhibit other bacteria.

In vivo assay

Infection and Treatment

The weights of all the animals were recorded prior to infection and all rats were confirmed to be healthy. The animals were randomly divided into 8 groups of 10 animals each. The rats in Group 1, the control, were not infected but received normal saline (0.4mL) while the other seven groups were infected animals each. The rats in Group 1, the control were not infected but received normal saline (0.4µL) while the other seven groups were infected animals each. The rats in Group 1, the control were not infected but received normal saline (0.4µL) while the other seven groups were infected animals each.

Clinical Examination

A modification of the method of Oladipo et al. (2014a; 2015b) was used for clinical examination of the experimental animals. The animals were weighed on a daily basis; blood samples were drawn from all the animals after three weeks of treatment and the blood was allowed to clot at room temperature and then centrifuged at 310 g for 12 minutes. The serum was collected and used for analysis, which included total protein, blood glucose, albumin, globulin, and enzymes activities (AST and ALT).

Serum Biochemical parameters Analysis

Glucose Analysis: The quantitative determination of serum glucose was carried out using commercially available diagnostic experimental kits purchased from Diagnosticom Limited (Budapest, Hungary).

Albumin Assay: Quantitative colorimetric albumin determination was carried out by using Albumin Assay kit which was purchased from BioSino Biotechnology & Science Inc. (China). Serum total protein was determined according to Lowry’s method. Total protein minus albumin equals globulin.

Transaminases Assay: The determination of AST and ALT was based on the fact that phenyl hydrazine was produced after incubating the substrate with the enzyme was measured spectrophotometrically. The amount of phenyl hydrazine formed was directly proportional to the enzyme quantity. Standard kits for the determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were obtained from Span diagnostics, Surat (Gujarat). Colorimetric procedure in which the oxaloacetate and/or pyruvate formed in either the AST or ALT reaction is combined with 2, 4-dinitrophenylhydrazine to yield a brown-coloured hydrazone which is measured at 505 nm.

Histological assessment of the gastric tissues and GIT

The animals were anesthetized by ketamine (12 mg kg⁻¹ body weight) followed by cervical dislocation for killing, stomachs and small intestines were removed and fixed in 10% formalin and embedded in paraffin. The sections (5µm) were cut with a microtome, stained with hematoxylin and eosin, and assessed under an Olympus microscope (Olympus Optical Co., GMBH, Hamburg, Germany). Images were captured using Camedia software (EZ2OP 5.0 Megapixel; Hamburg, Germany) at 20X magnification (Oladipo et al., 2014a; 2015b).

Statistical analysis

Statistical analysis of the data obtained was carried out using GraphPad Instat 3 software. Comparison between groups was made using one-way analysis of variance.

RESULTS AND DISCUSSION

Identification of bacterial strains

The isolates were found to be Gram’s positive rod, catalase, oxidase, methyl red, coagulase, urease and indole negative. They were unable to hydrolyze casein and gelatin but they were all able to grow at pH of 3.9 and 9.2. In this present study, six organisms isolated from food samples (ogi, kunnu, yogurt and palm wine) were characterized and identified to be Lactobacillus species using polyphasic taxonomy approach as described by Oladipo et al. (2013). The sequences were deposited in the GenBank database and accession numbers assigned to each strain as shown in Table 1.

Determination of bacteriocin production and antimicrobial spectra

Determination of the antimicrobial activity was carried out for all the Lactobacillus isolates and the bacteriocin produced showed antimicrobial inhibitory activity against Serratia marcescens, Micrococcus luteus, Bacillus cereus, Bacillus subtilis, Escherichia coli, Lactococcus lactis, Staphylococcus aureus and Bacillus pumilus but no activity was shown against Proteus mirabilis, Proteus vulgaris, Klebsiella pneumonia, Enterobacter cloacae and Pseudomonas aeruginosa. Lactobacillus plantarum MO21 and Lactobacillus plantarum MP12 had broad spectra of activities against the indicator organisms used (Table 2). The culture supernatant of Lactobacillus species was found to possess’ antimicrobial activity against Gram positive and Gram negative bacteria. This supports the previous findings of Nes et al. (1996) who reported that bacteriocins are proteinaceous compounds with inhibitory activity against more or less related bacterial genera. Juven et al. (1992) also reported that the ability of the Lactobacilli to produce metabolites such as bacteriocins has been suggested as being responsible for their ability to inhibit other bacteria.

In-vivo evaluation of the effects of the bacteriocinogenic strains and their bacteriocins

Table 3 shows the result of weight gained by the experimental rats during in-vivo assay. No death was recorded and their weights were measured on a weekly basis for three weeks. The control had the highest weight gained after the first, second and third week while the infected but not treated group showed the lowest weight gained. No changes in rats’ behavior, daily activity or physiology of the
experimental rats was observed and the weight gained was regular. The weight gain result reveals that the body weight of the rats increased during the 3 weeks of experiment. The low gained weight recorded in the untreated rats may be as a result of the infection induced by *E. coli* while the high gained weight recorded in the bacteriocin treated rats confirmed the findings of Fuller and Gibson (1997) who reported that bacteriocin has been used as growth promoters due to their ability to suppress the growth and activities of growth depressing micro flora and their ability in enhancing absorption of nutrients through the production of digestive enzymes.

Biochemical evaluation of sera

The result of the biochemical analysis showed an increase in the values of AST, ALT, albumin, total protein, globulin, cholesterol, bilirubin and glucose of the untreated rats due to the prolong infection caused by *E. coli*. But the bacteriocin treatment normalized the blood serum of the treated groups (Table 4). With regards to serum biochemical analysis, the high level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of the untreated group is an indication of liver damage or dysfunction caused by the administration of *E. coli*. Alanine aminotransferase (ALT) is principally found in the liver and is regarded as being more specific than AST for detecting liver cell damage (Johnston, 1999; Cheesborough, 1991). A rise in ALP activities has been linked with an increased osteoblastic activity (Baron et al., 1994) and lack of bile flow (cholestasis). Devaraj (2012) reported that when body tissue or an organ such as the heart or liver is diseased or damaged additional AST is released into the bloodstream. The lower AST and ALT values in rats treated with bacteriocin indicate liver function improvement brought about by bacteriocin treatment. As these parameters represent liver function, increase in their levels will indicate liver damage (Gad, 2007). Similar positive effect on the biochemical parameters of rats’ serum were also observed by other authors with different probiotic treatment (Fukushima and Nakan, 1995). Biochemical analysis showed high total protein, albumin, globulin, glucose, cholesterol and bilirubin in the rats from untreated group compared to the control and treated rats. Amdekar et al. (2010) reported that *E. coli* infection can cause the rise in total protein of blood serum and Koneko (1989), reported that increase in total protein and globulin in serum have been associated with bacteria septicemia and liver disease. The observed elevated bilirubin levels in the blood of infected rats may arise from free radical damage caused by the pathogen. This damage may be to the liver, red blood cells or the heart. Baranano et al. (2002) reported that elevated bilirubin levels in blood show risk of cardiovascular diseases, increased breakdown of red blood cells and liver failure. Koneko (1989) reported that increase in total protein and globulin concentration in serum has been associated with bacterial septicaemia and liver disease which was also observed in untreated rats. The lower Cholesterol level in the treated group when compared to untreated rats confirmed the ability of bacteriocin to function as anticholesterol substance. Casas and Dobrogosz (2000) said generally that Lactobacilli have anticholesterololic effect and Bertazzoni et al. (2001) found that Lactobacilli have direct effect on cholesterol levels by assimilation and removal from the growth medium.

Histological assessment of gastric tissues and GIT

The histopathology of the stomach showed that the *E. coli* infection caused disruption at the junction between the sub mucosal and muscular layer, infiltration by inflammatory cells but this was normalized by bacteriocin treatment as no visible lesions was observed in the stomach of the bacteriocin treated rats (Fig. 1). Also, the histopathology of the intestine of the untreated rats showed blunted and disintegrated villi tips and there were large parasitic sections along with little mucosal debris in the intestinal lumen which was cured by the bacteriocin treatment (Fig. 2). The result of the histopathology of the stomach of rats after three weeks of bacteriocin treatment showed no visible lesion as compared to the control. Also, the mucosa, submucosa and muscular layer were unaffected. The protection of the gastro intestinal tract was observed in the intestine of the bacteriocin treated rats because no visible lesions was observed and the serosa, villi and lumen was intact after three weeks of bacteriocin treatment. This indicated that bacteriocin treatment was able to cure the infection caused by the *E. coli* and this is in accordance with Oyetayo et al. (2003) whose histopathological result also confirmed the protective effect of the Lactobacillus. There has been a number of studies that reveal the probiotic potential of Lactobacilli as health promoting bacteria in man and animals (FAO/WHO, 2001). They play a major role in protecting the immune system against pathogens residing in the human body.

### Table 1 Lactobacillus species used in this study

<table>
<thead>
<tr>
<th>Name of isolates</th>
<th>Source of isolation</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus plantarum MO21</td>
<td>Ogi</td>
<td>KJ739519</td>
</tr>
<tr>
<td>Lactobacillus plantarum MP12</td>
<td>Palm wine</td>
<td>KJ739520</td>
</tr>
<tr>
<td>Lactobacillus casei MK21</td>
<td>Kannu</td>
<td>KJ739521</td>
</tr>
<tr>
<td>Lactobacillus casei MO11</td>
<td>Ogi</td>
<td>KJ739522</td>
</tr>
<tr>
<td>Lactobacillus brevis MK11</td>
<td>Kannu</td>
<td>KJ739523</td>
</tr>
<tr>
<td>Lactobacillus buchneri MY21</td>
<td>Yogurt</td>
<td>KJ739524</td>
</tr>
</tbody>
</table>

### Table 2 Antimicrobial activity of Lactobacillus species against indicator organisms

<table>
<thead>
<tr>
<th>Indicator organisms</th>
<th>L. plantarum MO21</th>
<th>L. plantarum MP12</th>
<th>L. casei MK21</th>
<th>L. casei MO11</th>
<th>L. brevis MK11</th>
<th>L. buchneri MY21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serratia marcescens</td>
<td>10.0± 0.12</td>
<td>11.0± 0.23</td>
<td>10.0± 0.11</td>
<td>9.0± 0.13</td>
<td>10.0± 0.12</td>
<td>9.5± 0.13</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>10.0± 0.30</td>
<td>11.0± 0.21</td>
<td>9.0± 0.14</td>
<td>11.0± 0.12</td>
<td>9.0± 0.23</td>
<td>10.0± 0.31</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>11.5± 0.25</td>
<td>10.5± 0.11</td>
<td>8.0± 0.13</td>
<td>12.0± 0.23</td>
<td>10.0± 0.15</td>
<td>11.0± 0.23</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>12.0± 0.10</td>
<td>11.5± 0.10</td>
<td>9.0± 0.11</td>
<td>9.0± 0.15</td>
<td>11.0± 0.16</td>
<td>8.5± 0.12</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>12.5± 0.12</td>
<td>12.0± 0.14</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactococcus lactis</td>
<td>12.0± 0.11</td>
<td>10.5± 0.13</td>
<td>10.0± 0.25</td>
<td>8.0± 0.31</td>
<td>8.5± 0.11</td>
<td>12.0± 0.24</td>
</tr>
<tr>
<td>Enterococcus clavae</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>13.0± 0.20</td>
<td>12.0± 0.12</td>
<td>11.0± 0.12</td>
<td>9.5± 0.16</td>
<td>9.5± 0.10</td>
<td>10.0± 0.20</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>9.0± 0.21</td>
<td>9.2± 0.22</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>10.0± 0.21</td>
<td>10.0± 0.23</td>
<td>9.0± 0.24</td>
<td>8.7± 0.22</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacillus pumilus</td>
<td>11.0± 0.22</td>
<td>10.5± 0.25</td>
<td>8.0± 0.26</td>
<td>8.5± 0.24</td>
<td>10.0± 0.24</td>
<td>13.0± 0.11</td>
</tr>
</tbody>
</table>

### Table 3 Weight gained by experimental rats during experimental period

<table>
<thead>
<tr>
<th>Weeks of treatment</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
<th>Group 7</th>
<th>Group 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>150± 0.47</td>
<td>150± 0.41</td>
<td>150± 9.81</td>
<td>150± 9.56</td>
<td>150± 0.47</td>
<td>150± 9.40</td>
<td>150± 0.47</td>
<td>150± 9.47</td>
</tr>
<tr>
<td>Week 1</td>
<td>158± 0.29</td>
<td>150± 0.22</td>
<td>152± 0.26</td>
<td>151± 0.22</td>
<td>152± 0.26</td>
<td>152± 0.27</td>
<td>151± 0.26</td>
<td>152± 0.27</td>
</tr>
<tr>
<td>Week 2</td>
<td>187± 1.41</td>
<td>153± 0.90</td>
<td>170± 1.47</td>
<td>171± 0.99</td>
<td>170± 0.79</td>
<td>173± 1.20</td>
<td>172± 1.00</td>
<td>173± 1.01</td>
</tr>
<tr>
<td>Week 3</td>
<td>200± 1.21</td>
<td>158± 0.89</td>
<td>190± 0.81</td>
<td>194± 0.92</td>
<td>196± 0.91</td>
<td>194± 0.99</td>
<td>192± 0.96</td>
<td>193± 1.09</td>
</tr>
</tbody>
</table>

Each value is a mean of 3 replicates ± standard deviation. – N.D. Not Detected. Diameter of zones of inhibition are in millimeter.
To their use as probiotics. The use of these microorganisms as probiotics has been extensively studied due to their potential benefits in terms of health. Lactobacillus casei, for instance, is known for its probiotic properties. It is often used in the treatment of various gastrointestinal disorders due to its ability to produce antimicrobial substances, such as bacteriocins, which help in the disruption of bacterial film on the surface of the gut and protect against infection. Additionally, the bacteriocin produced by Lactobacillus casei may affect the microorganisms of the oral cavity and protect against oral infections.

The use of these microorganisms as probiotics can positively contribute to the improvement of liver function, protection of the gastrointestinal tract, and the hygiene and safety of the food products produced. Therefore, the special characteristics of these microorganisms and their bacteriocins may be beneficial for biomedical purposes.

In Conclusion, the antimicrobial characteristics of the Lactobacillus casei species have a positive impact on its use as a starter culture for traditionally fermented foods. With a view of improving the hygiene and safety of the food products produced and the bacteriocin produced, it is responsible for an important effect in the disruption of E. coli plasma membrane and protection of the gastrointestinal tract. Therefore, the special characteristics of these Lactobacillus species can positively contribute to their use as probiotics. The use of these Lactobacillus species and their bacteriocins may be beneficial for biomedical purposes.

### Table 4 Biochemical analysis of the blood serum of experimental rats

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
<th>Group 7</th>
<th>Group 8</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>3.9±0.21</td>
<td>4.9±0.41</td>
<td>3.9±0.41</td>
<td>3.9±0.21</td>
<td>3.9±0.41</td>
<td>3.9±0.51</td>
<td>3.9±0.25</td>
<td>3.9±0.11</td>
<td>4.9±0.41</td>
</tr>
<tr>
<td>Glucose (g/dl)</td>
<td>282.1±23.20</td>
<td>292.8±23.20</td>
<td>280.1±40.31</td>
<td>292.8±23.20</td>
<td>292.1±23.20</td>
<td>292.8±23.20</td>
<td>292.1±22.31</td>
<td>282.2±20.49</td>
<td>304.6±45.40</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>67.0±16.00</td>
<td>62.0±29.00</td>
<td>67.0±16.00</td>
<td>60.0±27.00</td>
<td>63.0±14.00</td>
<td>63.0±23.00</td>
<td>49.0±9.30</td>
<td>71.0±23.00</td>
<td>78.0±9.61</td>
</tr>
<tr>
<td>Cholesterol (g/dl)</td>
<td>49.0±9.30</td>
<td>29.0±14.00</td>
<td>49.0±9.30</td>
<td>60.0±27.00</td>
<td>63.0±14.00</td>
<td>63.0±23.00</td>
<td>49.0±9.30</td>
<td>71.0±23.00</td>
<td>78.0±9.61</td>
</tr>
<tr>
<td>Bilirubin (g/dl)</td>
<td>70.3±9.81</td>
<td>70.3±9.81</td>
<td>70.3±9.81</td>
<td>70.3±9.81</td>
<td>70.3±9.81</td>
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<td>70.3±9.81</td>
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</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>0.72±0.10</td>
<td>0.72±0.10</td>
<td>0.72±0.10</td>
<td>0.69±0.18</td>
<td>0.62±0.21</td>
<td>0.67±0.04</td>
<td>0.62±0.10</td>
<td>0.72±0.10</td>
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</tr>
<tr>
<td>AST (IU/l)</td>
<td>55.0±8.30</td>
<td>62.0±29.00</td>
<td>67.0±16.00</td>
<td>60.0±27.00</td>
<td>63.0±14.00</td>
<td>63.0±23.00</td>
<td>49.0±9.30</td>
<td>71.0±23.00</td>
<td>78.0±9.61</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>62.0±29.00</td>
<td>67.0±16.00</td>
<td>60.0±27.00</td>
<td>63.0±14.00</td>
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<td>49.0±9.30</td>
<td>71.0±23.00</td>
<td>78.0±9.61</td>
<td>78.0±9.61</td>
</tr>
<tr>
<td>Biliverdin (g/dl)</td>
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<td>0.62±0.21</td>
<td>0.67±0.04</td>
<td>0.62±0.10</td>
<td>0.72±0.10</td>
<td>0.72±0.10</td>
<td>0.72±0.10</td>
<td>0.72±0.10</td>
<td>0.72±0.10</td>
</tr>
<tr>
<td>Group 1</td>
<td>Group 8</td>
<td>Group 3</td>
<td>Group 4</td>
<td>Group 5</td>
<td>Group 6</td>
<td>Group 7</td>
<td>Group 8</td>
<td>Untreated</td>
<td></td>
</tr>
</tbody>
</table>

Each value is a mean of 3 replicates ± standard deviation.

### Reference


