EFFECT OF ALKALOID EXTRACT OF *PHYLANTHUS NIRURI* ON RABBITS INFECTED WITH ENTEROPATHOGENIC *ESCHERICHIA COLI*

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Abstract

Five groups of six rabbits each were infected with $10^6$ cell/ml inoculums of “enteropathogenic” *Escherichia coli* and treated with alkaloid extract of *Phyllanthus niruri* administered as straw-berry at 100mg/day divided in four doses. Sample of serum and urine were collected before infection, 26hr after infection, 3rd day of therapy (72hr) and 48hr after 7th day of therapy. Creatinine, urea nitrogen, aspartate transaminase, alanine transaminase packed cell volume, total white blood cell count, polymorphonuclear neutrophils, lymphocytes and levels of hemoglobin were analyzed in serum while alkaloid concentration were analyzed in both samples. There were no changes in enzymes concentration. A decrease in PCV, hemoglobin lymphocytes and an increase in TWBC and neutrophils were noticed. Alkaloid was well tolerated and showed a great promise in therapy.

KEYWORDS: Alkaloid, Blood urea nitrogen, Enteropathogenic *Escherichia coli*, *Phyllanthus niruri*, aspartate transaminase, alanine transaminase.

INTRODUCTION

The various serotype of *Escherichia coli* that causes diarrhea are classified according to their virulence determinants and these imbues the pathotypes with the capacity to cause clinical syndromes with distinctive symptoms [1]. For example enteropathogenic *E. coli* (EPEC) causes non-specific gastroenteritis especially in children in developing countries [1]. EPEC also differ from other pathotypes of *E.coli* in that it typically carries an EPEC adherence factor plasmid. These plasmids encodes bundle-forming pili (*Bfp*) which promotes bacterial adherence to epithelial cells and are an essential virulence determinant [2] and a transcriptional activator, *per* that up regulates genes within a chromosomal pathogenicity island.
termed the locus of enterocyte effacement [1]. This pathogenicity island encodes a number of essential virulence proteins, including the surface protein intimin, which is required to produce the attaching-effacing lesions that are a key feature of EPEC-induced pathology. Pathogenic isolates of E.coli have a relatively large potential for developing resistance [3] and report of multidrug resistance are not infrequent [4]. Before the advent of modern medicine of which many drugs were synthetically produced, extract of many plants were known to elicit certain reactions in human body when applied in a prescribed manner. Among such plants is Phyllanthus niruri L., (syn P. fraternus Webster). It belongs to the Euphorbiaceae family and has been claimed to be an excellent remedy for jaundice and hepatitis [5]. Based on its long documented history of uses in the Amazonian region, the plant is considered analgesic and as aperitif, carminative, digestive, emmanagogue, laxative, stomachic tonic [6]. It is also believed to be helpful in treating edema, anorexia and diabetes [7].

Many of the active constituents found in the plant are biologically active lignands, glycosides, flavonoids, saponins, alkaloids, ellagitannins and phenylpropanoids [8], common lipid sterols and flavonoids also occur in the plant [9]. Alkaloids are organic nitrogen containing compound found in 20%-30% of vascular plants [10] and at lower doses are useful pharmacologically. Morphine, codeine, atropine and ephedrine are just a few of the plant alkaloids currently used in medicine [11]. Other alkaloids, including cocaine, nicotine and caffeine, enjoy a widespread non-medical use as stimulants or sedatives [10, 11]. Some alkaloids are medically useful for the cure of human diseases e.g. atrophies in treatment of bronchial asthma [10]; intestinal and biliary colic, and to dilate pupils of the eye [11]. The purpose of this work is to investigate the in vivo effectiveness of alkaloid extracted from P.niruri; previously discovered to be potent in vitro on EPEC and to study its tolerance and toxicity in rabbits infected with this bacterium.

Table 1: Identification of Escherichia coli (EPEC) from stool and urine samples after infection

<table>
<thead>
<tr>
<th>Sample</th>
<th>Colony</th>
<th>Grams reaction</th>
<th>Fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Characteristic</td>
<td>Indole</td>
<td>Lactose</td>
</tr>
<tr>
<td>Stool</td>
<td>circular raised -ve rods</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Urine</td>
<td>circular raised -ve rods</td>
<td>+ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>
MATERIALS AND METHODS

Collection of Plant Materials

*Phyllanthus niruri* was collected from farmlands at Ado-Ekiti in Nigeria between the months of July and September, 2007 and identified in the herbarium of the Department of Plant Science University of Ado-Ekiti, where a voucher specimen No FPA 206 was deposited.

Group of rabbits

Thirty rabbits each divided into five groups of six were reared for five months for this purpose in the animal’s house of the Science Technology Department Federal polytechnic, Ado Ekiti.

Extraction of Crude Alkaloid

The method of Naik and Juvekar [11] was employed for the extraction. A 15g portion of the milled plant material was macerated in 100ml of 95% ethanol and allowed to stand for three days. The ethanol solution was filtered and evaporated under reduced pressure with Bibby rotary evaporator (EVF-530-010K) and the residue suspended in 30ml distilled water. 20ml chloroform was added and passed through a Whatman No 4 filter paper. The extract was dried and 10% sodium sulphate was added, filtered and evaporated under reduced pressure to obtain a light brown powder.

Sources of Test Bacterium

Enteropathogenic *Escherichia coli* (EPEC) isolated from clinical specimens of diarrheogenic *E. coli* (DE) and identified by polymerase chain reaction (PCR) and southern hybridization [12] was reactivated from stock in the Microbiology laboratory of the Department of Science Technology, Federal Polytechnic, Ado-Ekiti.

Bacteriological Assay

Stool and urine samples were collected from six rabbits in each group and cultured on blood and MacConkey agar (Oxford Ltd, Basingstoke, UK) before five of it was fed with yoghurt contaminated with 0.5ml bacterial (10^8 cells/ml) suspension of EPEC. After 24hrs, specimen of stool and urine were collected for culture from five rabbits and treatment by administrating of alkaloids commenced after 48hrs. Alkaloid was administered as an oral-straw-berry at a dose of 100ml/day (divided in four doses) to the rabbits. Tolerance and toxicity studies were adopted from Monica [17] and included white blood cell count (WBC), packed cell volume (PVC), hemoglobin (Hb) analysis and Tom [21]. Blood urea nitrogen (BUN), aspartate transaminase (AST), alanine transaminase (ALT) analyzed before the administration of alkaloid, on the third day of therapy and 48hrs after the 7th day of therapy. The specimen on the third day was routinely taken 2hrs after the morning dose.

Crude alkaloid was measured in the serum and urine with spectrophotometric method [20]. The stool and urine samples of the rabbit were collected and cultured on Blood and MacConkey agar incubated at 37°C for24hrs. Growth was identified 48hrs after the 7th day of therapy.
RESULTS AND DISCUSSION

Six rabbits in each group with enteropathogenic *E. coli* (EPEC) infection was screened and pathogenic *E. coli* were recovered in the stool and urine of all the infected rabbits (Table 1). One of the rabbits in each group (the 6th) was not included for technical reasons. The mean results of alkaloid effect on the concentration of the creatinine, urea nitrogen, and aspartate transaminase, alanine transaminase, packed cell volume, total white blood count, neutrophils lymphocyte and hemoglobin in the body fluid are summarized in Table 2.

There is no variation in the values of enzymes on the 1st, 3rd, and 9th day of therapy as compared to the control rabbit. The PVC decreases from 35% to 30.6%, 30.4% and 33% on the 1st, 3rd, and 9th day respectively. The TWBC increased from 8.2 x 10^9/L to 17.2 x 10^9/L, 14.5 x 10^9/L and 13.3 x 10^9/L on the 1st, 3rd, and 9th day respectively while neutrophil also increased from 55% to 81.6%, 72.5% and 57.4% on 34 these respective days. The lymphocyte decreased from 45% to 81.1%28.0% and 42.2% on the 1st, 3rd and 9th day respectively and the hemoglobin value decreased from

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**Table 2: Comparison of the mean values of serum and enzyme analysis on days 1, 3 and 9**

<table>
<thead>
<tr>
<th>Test</th>
<th>Days</th>
<th>Control</th>
<th>1</th>
<th>3</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dl)</td>
<td></td>
<td>0.11</td>
<td>0.11</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>BUN (mg/l)</td>
<td></td>
<td>0.1</td>
<td>0.1</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>AST (u/l)</td>
<td></td>
<td>31</td>
<td>31</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>ALT (u/l)</td>
<td></td>
<td>31</td>
<td>31</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>PVC (%)</td>
<td></td>
<td>35</td>
<td>30.6</td>
<td>30.4</td>
<td>33</td>
</tr>
<tr>
<td>TWBC (x10^9/l )</td>
<td></td>
<td>8.2</td>
<td>17.2</td>
<td>14.5</td>
<td>13.3</td>
</tr>
<tr>
<td>N (%)</td>
<td></td>
<td>55</td>
<td>81.6</td>
<td>72.5</td>
<td>57.4</td>
</tr>
<tr>
<td>L (%)</td>
<td></td>
<td>45</td>
<td>18.1</td>
<td>28.0</td>
<td>42.2</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td></td>
<td>11.7</td>
<td>10.5</td>
<td>10.3</td>
<td>11.7</td>
</tr>
</tbody>
</table>
11.7g/dl to 10.5/dl, and 11.3g/dl on the respective days of therapy. The mean levels of crude alkaloid in serum of the rabbits are indicated in figure 1. The concentration reduced from the 1st to the 9th day of therapy in the five groups of rabbits while the mean percentage of alkaloid in urine collected between the 1st and 3rd day were 39.0mg/ml, 47.7mg/mL, 49.7mg/ml, 53.5mg/ml and 53.8mg/ml in the respective groups (Fig. 2).

![Fig 1: Mean value of crude alkaloid in serum (mg/ml) on 1st, 3rd, 6th and 9th day of administration](image)

After the first day of infection (24h), the rabbits showed symptoms of diarrhea, fur collapse and low appetite; signs and symptoms of EPEC infection [12, 19]. The packed cell volume decreased due to the destruction of red blood cells as a result of the infection. On the other hand, the total white blood cell count and the neutrophils increased due to the infiltration of polymorpho-nuclear leucocytes induced by the infection. They are phagocytes which are actively involved in the processing of antigenic substances for onward destruction by therapeutic agents [13, 14]. They move in large numbers to the point of infection and exhibits phagocytic action. Lymphocytes are affected adversely therefore it decreases and the value of hemoglobin also decrease due to destruction of the red blood cells. Our findings were in agreement with previous reports of high percentage of polymorpho-nuclear neutrophils during bacterial infection [1].

After three days of therapy, the PCV, hemoglobin, and lymphocytes increased slightly while TWBC and neutrophils decreased. This is an indication of positive response, to treatment. On the 9th (48hrs after therapy), the previously observed symptoms of diarrhea stopped, fur on the skin of the rabbits takes its normal appearance and the rabbits gain their appetite. The culture of urine of the rabbits showed no growth while in stool sample, EPEC was not isolated. It was observed that before and during the cause of the administration of the alkaloid, there was no change in the concentration of BUN, blood creatinine, AST and ALT. This implies that the liver, kidney, heart, pancreas and muscles were not adversely affected. This supports antimutagenic, antispamotic and antitoxoigenic properties reported by Santos et al. [14] and also the antitumor, antimalarial, analgesics, tranquilising and reknown potent pharmacological activities of alkaloid reported by Denni and Hussain [15] and Sharma and Gupta, [20].
The elimination of alkaloid from the body of the rabbit was independent of dose, its concentration in serum versus days of therapy was proportional to the amount of alkaloid absorbed and the principle of superposition applies i.e, the ratio of plasma concentration to the amount of alkaloid absorbed versus day of therapy was independent of dose (Figure 1&2). These findings substantiate the constancy of absorption, distribution, metabolism and excretion with each administration of the alkaloid and it correlates with the report of Naik and Juvekar [11]. Hence, alkaloid extract of P. niruri has shown a great promise in the treatment of intractable infectious disease of diarrhea caused by enteropathogenic E.coli.

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