Effects of *Bidens Pilosa (L)* Extract on Haematological Parameters of Swiss Albino Rats Orogastrically Dosed with *Escherichia coli* O157:H7


Abstract—A large number of medicinal plants and their purified constituents have been shown to have beneficial therapeutic potentials. In this study, ethanolic extract of *Bidens pilosa* was evaluated for its invivo activity on haematological parameters in Swiss albino rats orogastrically dosed with *Escherichia coli* O157:H7. Fifteen Swiss albino rats were used for the study. The animals were divided into five groups of three rats each. The first, second and third group of rats were orogastrically dosed with 9.1 × 10^5 cfu/ml of *E. coli* O157: H7 to induce infection. The first group was treated with 500 mg/kg Body weight (Bw) of the ethanolic extract of *B.* pilosa, the second group was treated with Ofloxacin (16mg/kg Bw), while the third group was not treated. The fourth group was given only the plant extract, while the fifth group was given sterile distilled water. The results of the haematological assay indicated that: the infected-untreated rats showed lowest mean values of PCV (34.00±2.50%), RBC (6.54±0.45%) and HB (11.50±0.83%); and highest ESR (4.50±0.50%). In the infected-extract-treated group, a significant increase in the PCV (45.00±1.00%) and HB (15.00±0.33%) was observed. The group fed with extract alone had the highest mean values of PCV (51.00±1.00%), RBC (11.10±0.95%) and HB (17.00±0.33%). Similar pattern was observed for the results obtained for the white blood cell differential count. The infected-extract-treated group, and the group to which only extract was administered without infection showed significant increase in lymphocyte count (61.00±1.00%) and (73.50±2.50%) respectively. Conversely, the infected-untreated group showed a decline in lymphocyte count (54.50±3.50%). The results obtained from this study revealed that ethanolic leaf extract of *Bidens pilosa* exhibited haematopoietic potential and tends to modulate the values of White Blood Cell differential count in Swiss albino rats.

Index Terms—*Bidens pilosa*; *Escherichia coli* O157:H7; Haematological parameters; Swiss albino rats.

I. INTRODUCTION

Haematological parameters are useful indices that can be employed to assess the physiological and pathological status of an animal [1], [2]. Blood is a composite of erythrocytes, leucocytes, thrombocytes and plasma. It accounts for 7% of human body weight [3]. Oxygen distribution to the periphery from the lungs through the pulmonary capillaries, removal of carbon dioxide from the tissues back to the lungs through the systemic capillaries and maintenance of acidic and basic values of the body are the three main functions of erythrocytes [4]. Leucocytes are the main cells of the immune system that provide innate and specific adaptive immunity. They are further divided into five different classes which include; basophils, neutrophils, eosinophils, lymphocytes and monocytes [5]. Platelets play a major role in hemostasis, thrombosis, clot retraction, vessel constriction and repair, inflammation including promotion of atherosclerosis, host defense and even tumor growth/metastasis [6].

A large number of medicinal plants and their purified constituents have been shown to have beneficial therapeutic potential [7]. *B. pilosa* is a edible plant belonging to the Asteraceae family. It is widely distributed across different regions of the world [8]. All parts of the plant (leaves, flowers, seeds, and stems) are used as ingredients in folk medicine [9], [10], [11]. The pharmacological properties of the plant seems to be associated with the bioactive phytochemical compounds present in the plant [12]. Generally, this plant is applied as dry powder or tincture when used externally, and as maceration, or decoction when used internally [13]. *B. pilosa* either as a whole plant or different parts, has been reported to be useful in the treatment of more than forty diseases/disorders such as inflammation, immunological disorders, digestive disorders, infectious diseases, cancers, colitis, diarrhea, metabolic syndrome, wounds, and many others [14], [15], [11]. This study evaluated the effect of *B. pilosa* ethanolic extract on the haematological parameters of Swiss Albino rats.

II. MATERIALS AND METHODS

A. Test Bacterium

The organism was identified by its characteristic growth on sorbitol MacConkey agar (SMAC). The colonies were further confirmed using slide agglutination test with *E. coli* O157:H7 antiserum according to the method of [16].

B. Processing and Extraction of Bidens pilosa

Fresh leaves of the plant (*Bidens pilosa*) were air dried for four weeks until fully crispy, the leaves were pounded using clean mortar and pestle, then pulverized into fine powder by blending in a high-speed blender. They were kept separately in an airtight container to avoid moisture absorption. 200g of the powdered sample were soaked in 1500 ml of ethanol as solvent to extract the bioactive compounds. The container was left for 72 hours. After this period, it was sieved using muslin cloth and then filtered using no 1 Whatmann filter paper. The filtrate was vaporized to dryness using rotary

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evaporator. The extract was preserved in a sterile bottle at 4°C ready for use.

C. Experimental Animals and Diet

Fifteen (15) Swiss albino rats consisting of 9 females and 6 males were used for the study. The animals were divided into five groups of three rats each based on their sex. They were fed with standard rodent diet and acclimatized for a period of seven days prior to the experiment.

D. Determination of Infectivity Dose

From an overnight bacteria cultured in nutrient broth, the cells were harvested and washed by centrifuging at 3,000rpm for 5 minutes three times using sterile distilled water. The supernatant was carefully decanted and the cells transferred into 100ml sterile distilled water to make the stock cell culture. A sterile dilution was then made in test tubes containing 9ml of sterile distilled water from which 1ml was taken from each of the different concentrations already prepared in the test tubes to infect the experimental animals. A volume of 1ml was taken to determine the colony forming units using pour plate method. The dilution that produced the signs and symptoms in 50% of the animals given was taken as the infectivity dose ID50 of the organism [17].

E. Infection and Treatment

The first, second and third group of rats were infected with the test organism (E. coli O157: H7). The first group was treated with 800mg/kg Bw of the ethanolic extract of B. pilosa, the second group was treated with Ofloxacin (16mg/kg Bw), while the third group was infected but not treated. The fourth group was given only the plant extract, while the fifth group was given sterile distilled water.

F. Blood Collection

Anaesthetization method as described by [18] was used with slight modification in collection of blood from the rats used in this experiment. The rats’ blood were collected through cardio-vascular puncture. The blood collected from the experimental rats was carefully transferred into EDTA bottles for haematology tests.

III. RESULTS AND DISCUSSION

Infectivity Dose on Swiss Albino Rats

The swiss albino rats for each group (1 to 3) were orogastically dosed with an infectivity dose of 9.1 × 10^4 cfu/ml of the test bacterium.

Haematological Assay of Swiss Albino Rats

The result of the haematological assay of the albino rats infected and treated with the extracts showed that the extract caused a significant increase in the packed cell volume (PCV) 45.00±1.00 (group 1), while the infection caused a significant reduction in the PCV 34.00±2.50 (group 3) - this result is shown in table I. The same pattern was observed for the result obtained for the white blood cell differential count. The infection caused a decrease in the Lymphocyte count while causing an increase in the Neutrophils (30.00±1.00) and Monocytes (15.50±1.50) respectively. However, there is no significant decrease or increase in the Eosinophils and Basophils respectively as shown in table II.

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05).

Legend: LYMP= lymphocytes, NEUR= neutrophils, MONO= monocytes,

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<th>RBC</th>
<th>WBC</th>
<th>HB</th>
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Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05).

Legend: LYMP= lymphocytes, NEUR= neutrophils, MONO= monocytes, EOSIN= eosinophils, BASO= basophils, 1= infected with E. coli O157:H7 treated with extract, 2= infected with E. coli O157:H7 treated with Ofloxacin, 3= infected with E. coli O157:H7 but not treated, 4= given extract only, 5= control

IV. DISCUSSION

Haematological parameters are important in establishing the body’s functional status. Red blood cell and factors relating to it are major indices for evaluating circulatory erythrocytes, these entities are significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow’s capacity to produce RBC particularly in mammals [19], [20]. The onset of infection induced by E. coli O157:H7 in the experimental rats was signaled by fatigue, loss of appetite, dull furs and unformed stool. These signs and symptoms observed were in accordance with those described by [21]. The infection caused a decrease in PCV, RBC and HB; and an increase in ESR in the infected-untreated rat group. This might be due to the fact that pathogenic organisms cause acute inflammation and haemolysis [22], [23], [24] reported that “Surface-exposed proteins of Gram-negative bacteria are represented by integral outer membrane beta-barrel proteins and lipoprotein”. The complexity of the outer membrane of E. coli which functions as the selective permeability barrier surely contributes to its resistance mechanism against chemotherapeutic agents as it protects the cell from harmful chemicals, including detergents and antibiotics; making its treatment difficult [24].

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Compared with the infected-untreated rat group, those in group 1 (infected-extract-treated) and group 4 (uninfected but given extract only) showed significant increase in their PCV, RBC and HB, indicating the plant extract may contain active agents which promote the action of erythropoiesis (the humoral regulator of red blood cell production), and consequently increase production of erythrocytes [25], this finding is in agreement with the reports of [26] who found some medicinal plants which included: Phyllanthus emblica, Spinacia oleracea, Ficus carica, Phoenix sylvestris, Boerhavia diffusa, Aegle marmelos, Spinaca oneracean, Ficus carica, Phoenix sylvestris, Anthocleista grandiflora stem bark”. (2012) “Phospholipon 90G based SLMs loaded with ibrupofen: An oral anti-inflammatory and analgesic delivery mediated by hemoglobin – inflammatory effects of the plant. This may increase P. Pilosa significant increase in the lymphocytic count due to infections [31].

The major functions of the white blood cell and its differentials are to defend the body against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response [28]. The extract tends to modulate the values of WBC differential counts in the treated rats groups. The infected-extract-treated group and the group given extract alone showed significant increase in lymphocyte count, compared to the infected untreated group which showed a decrease in lymphocyte count. This result corresponds with earlier findings of [29], who reported that administration of methanol extracts of Terfairyia occidentalis to wistar rats stimulated increase in lymphocytic count; and [30], who reported that aqueous extract of the leaves of Erigeron floribundus stimulated increase in total lymphocytes of rabbit blood. The significant increase in the lymphocytic count due to B. Pilosa extract reflects the lymphopoietic and possible immunomodulatory effects of the plant. This may increase the animal’s resistance to infections [31].

V. CONCLUSION

The results obtained from this study revealed that ethanolic leaf extract of Bidens pilosa exhibits haematopoietic potential and tends to modulate the values of White Blood Cell differential count in Swiss albino rats.

REFERENCES


Oluyele Olumide is a Medical Microbiologist exploring studies on developing novel antimicrobial agents from natural sources, immunoregulation and immunity against infectious diseases- with notable publications to this effect. He is a researcher at Adekunle Ajasin University, Akungba Akoko, Nigeria and also a member of the American Society for Microbiology (ASM) and the Society for Applied Microbiology (SFAM).