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C-reactive Protein and Immunological Indices of Lectin Extract from Edible Fungus (*Pleurotus tuber-regium*) on Wounded Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Lectins are proteins/glycoproteins of non-immune origin with wide distribution in nature with the ability to agglutinate erythrocytes and recognize glycoconjugates. The study aimed at determining the healing effect of lectin extract from *Pleurotus tuber- regium* on acute wounds induced in male albino rats. Ten grams of the powdered edible mushroom of *Pleurotus tuber-regium* were used to extract lectin via the dialysis method. A total of 45 albino rats randomized into 9 groups of 5 rats each into Category A and B groups, and the blood sample was collected by ocular puncture for the category A after 10 days, while cardiac puncture for the Category B group after 28 days. The collected blood sample was analyzed for the various immunological parameters, using the quantitative ELISA technique for C-reactive protein, and Mindray BC-200 machine for full blood count. At negative control, the WBC levels and the neutrophils were increased for the Category B

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group to 19.79±1.22/L and 3.26±0.25%. A 2mg/kg lectin oral administration had 4.72±0.14% neutrophils for Category A group, while Category B group topical application, the highest value at 8mg/kg lectin had 4.98±0.52%. Both 36 mg/kg amoxicillin application and oral administration of lectin generated factors as C3b, and Histamine ($H^1$and H^2) receptors on eosinophils by chemotaxis attraction. With Category B group, 8 mg/kg lectin topical had the highest value of 21.30±2.17% eosinophils, and the difference in Category B group compared to the normal control at 17.64±1.1 81% was significant $p \leq 0.05$ and the rats would be showing signs of allergic responses. Both the oral and topical lectin applications to the wounded rats had higher platelet levels than the negative control in both Categories A and B groups indicating that lectin mobilized more platelet factors than the negative control group. With negative control, the CRP levels as a marker of inflammation rose to 3 times its normal folds in Category A group with 7.58±0.85ng/L and 6.06±0.85ng/L for Category B group. On 36 mg/kg amoxicillin administration to the rats from zero week to the 3rd week, there was a decrease in weight at 22.4% compared to the normal control of 31.3%. Both oral and topical administrations showed an increase in weight with 26.4% for 8 mg/kg oral administration and 27.3% for 4 mg/kg topical administration. Both 36 mg/kg amoxicillin and 8 mg/kg lectin oral administration had a two weeks period for the complete healing of the wounded rats. Oral administration of lectin to the wounded rats was nutritional and added weight to the rats.

Keywords: Lectin; pleurotus tuber-regium; dialysis method; hemagglutination assay; immunological parameters; c-reactive protein; acute wound healing; male albino rats.

1. INTRODUCTION

Lectins are proteins/glycoproteins of non-immune origin, which are widely distributed in nature [1]. They have at least one non-catalytic domain which binds reversibly to specific monosaccharide or oligosaccharide. Lectins recognizes sugar moieties in cell walls or cell membranes, alter the membrane physiology and trigger biochemical changes in the cell [2]. Food sources that contain lectin includes beans, peanuts, lentils, tomatoes, potatoes, eggplant, fruits, vegetable, legumes and wheat [3,4]. They are abundantly found in plants, animals and many other species and are known to agglutinate various blood groups of erythrocytes [5,3,4].

Lectins display an array of functions such as antifungal, anti-neoplastic, anti-inflammatory, anti-tumor, immunomodulatory, anti-insect and anti-viral activities [3,6,7]. Fungi have a large repository of lectins in which 82% of the lectins identified are from mushrooms [8,6]. Lectin extraction is usually achieved using different methods of diffusion in aqueous solution/desalting of proteins by dialysis [9], gel filtration and ion-exchange chromatography [10-12].

The ability to agglutinate cells distinguishes these proteins from other macromolecules, they have the ability to bind carbohydrates, in addition their non-immune origin differentiates them from anti-carbohydrate immunoglobulins that agglutinate cells [3]. Mushroom contains relatively large amount of carbohydrates, fiber and possess large number of nutritional, medicinal and pharmacologically important bioactive compounds including ribosome inactivating proteins, proteases, antifungal proteins and lectins [13,14]. Agglutination involves clumping of cells or inert particles by specific antibodies to surface antigenic components or to antigenic components absorbed or chemically coupled to red blood cells or inert particles [15].

Neutrophils are a type of white blood cell that led the immune system's responses and are the most abundant type, making up 55 to 70 percent of the white blood cells**.** They are formed from stem cells in the bone marrow and differentiated into subpopulations of neutrophil-killers and neutrophil-cagers. They are short-lived and highly mobile, as they can enter parts of tissue where other cells/molecules cannot. Neutrophils may be subdivided into segmented neutrophils and banded neutrophils (or bands). They are a type of phagocyte and are normally found in the bloodstream. During the beginning (acute) phase of inflammation, particularly as a result of bacterial infection, environmental exposure [16], and some cancers [17], neutrophils are one of the first responders of inflammatory cells to migrate toward the site of inflammation. The average lifespan of inactivated human neutrophils in the circulation has been reported by different approaches to be between 5 and 135 hours [18]. The short lifetime of neutrophils minimizes propagation of those pathogens that

parasitize phagocytes because the more time such parasites spend outside a host cell, the more likely they will be destroyed by some component of the body defence. Also, because neutrophil antimicrobial products can also damage host tissues, their short life limits damage to the host during inflammation [19].

Platelets, or thrombocytes, are small, colorless cell fragments in our blood that form clots and stop or prevent bleeding [20]. Platelets are made in our bone marrow, the sponge-like tissue inside our bones. Bone marrow contains stem cells that develop into red blood cells, white blood cells, and platelets. Low platelet concentration is called thrombocytopenia and is due to either decreased production or increased destruction. Elevated platelet concentration is called thrombocytosis, and is either congenital, reactive (to cytokines), or due to unregulated production of one of the myeloproliferative neoplasms or certain other myeloid neoplasms. A disorder of platelet function is a thrombocytopathy. Platelets have central role in innate immunity, initiating and participating in multiple inflammatory processes, directly binding pathogens and even destroying them [21].

This study aimed at the extraction of the lectin from *Pleurotus tuber-regmin* and subsequently administered orally in appropriate doses to the induced wounded male albino rats. Hence, to ascertain its biochemical and immunological effects on the levels of CRP, platelets, neutrophils, WBC and eosinophils on the clinical status in the induced wounded male rats within 4-week period of treatment.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

The *Pleurotus tuber-regium* stem (osu), was purchased from New market Aba, Abia State. Four *Pleurotus tuber-regium* stems with grown sporangiophores were purchased. The mushrooms were identified as edible *Pleurotus tuber-regium* by a taxonomist in the Department of Botany Nnamdi Azikiwe University Awka. The mushrooms were weighed and later kept in an incubator for four days to obtain the dry weight.

2.2 Cultivation Process

The cultivation process for the sporulation of the stem (osu) proceeds as distilled water was used for sprinkling on the plastic containers to quicken sporulation. The stem (osu) was earlier washed in distilled water and buried in plastic containers or on polyethylene bags filled with washed sand in 0.1M H_2SO_4. It takes one to two months before full sporulation of the osu stem.

2.3 Extraction of Lectin

The mushroom was grinded using an electric blender after which 10g of the powdered mushroom was weighed out and homogenized in 200 ml of phosphate buffer saline (pH 7.4) and kept at 4^oC shaker incubator for overnight.

Following the homogenization of the mushroom, the supernatant was transferred into several test tubes and were centrifuged for 30mins at 4000 revolution per second. After the centrifugation, the supernatants were collected as the crude protein extract and were precipitated by salting out process using ammonium sulphate at 60% saturation for 10 minutes.

The dialysis bag was soaked for some minutes inside a beaker containing distilled water. Thirty miles each of the samples was measured out into two different dialysis bags, the two ends of each bag were tied to avoid the escape of the sample into the phosphate buffered saline. The bags were soaked inside a beaker that contains phosphate buffered saline and placed on a shaker incubator which was set on speed 3500rev/mins, and the phosphate buffered saline was changed every 2hours.

2.4 Desalting of Proteins by Dialysis [9]

Principles: Dialysis is commonly used for removing salts from the proteins. The presence of salts in proteins interferes in many ways. Special semi permeable membrane called dialysis tubes have the property to allow compounds with small molecular weight to pass through them but not those with high molecular weight like proteins which are held back. The protein solution to be desalted is taken inside a dialysis bag and the two ends are secured tightly to prevent leakage.

Water freely permeable would have entered the bag and diluted the protein also. There are several methods of concentrating this solution like lyophilization and Ultra filtration under vacuum. But a very simple procedure is to carefully bury the dialysis bag with solution in a jar containing sucrose or polyethylene glycol and kept in a refrigerator. Water will move out and get absorbed by sucrose or polyethylene glycol.

2.5 Protein Determination with the Folin Reagent

Principle: This method is an extension of the biuret procedure for protein determination as described by Lowry et al. [22]. The formation of a copper-protein complex in alkaline solution reduces a phosphomolydie – phosphotungstate reagent to yield an intense blue colour and is the basis for this method. It estimates the tyrosine and tryptophan content of protein with a sensitivity that is 10 to 100 times superior to UV-absorption method for protein estimation at 280nm and biuret methods respectively. It is however subject to interference by K^+, Mg^+, NH^{4+} EDTA, TRIS, carbohydrates, 2-mercaphtoethanol and dithiothreitol among others.

2.6 Materials

4% Na_2 CO_3 anhydrous, 0.2N NaOH, 1% $CuSO_4 5H_20$, 2% Naktartrate, Folin-Ciocalteu reagent, bovine serum albumin standard; 200µg/ml

2.7 Reagents

Solution A: 2% Na_2CO_3 in 0.1N NaOH-.mix equal volumes of 4% Na_2CO_3 anhydrous solution and 0.2N NaOH. Solution B 0.5% $CuSO_4$ $5H_2O$ in 1% Naktartrate. Solution C; Mix 50ml of solution A with 1ml of solution B. Solution D, Folin-Ciocalteu reagent diluted with two volumes of water. Solution A and B are not stable and must be prepared freshly from the stock reagent.

2.8 Procedure

Label six test tubes in addition to at least two duplicate tubes for each example to be estimated. Dispense standard bovine serum albumin into the test tubes, followed by addition of saline or distiller water to make up to 0.5ml. Add 2.5mL of solution C (freshly prepared) with thorough mixing and allow to stand for 10mins. Then add 0.25ml solution D to all tubes with thorough mixing. Let the mix stand for 30mins before reading absorbance at 750nm. All test samples are prepared as the standards. Plot absorbance against concentration (mg/mL) for standard bovine serum albumin tests and extrapolate absorbance values of unknown test samples to concentration from standard curve.

2.9 Dubois Method of Carbohydrate Estimation

Principle: The method depends on the colour reaction between phenol and concentrated H_2SO_4 and simple sugars, oligosaccharides, polysaccharides, and their derivatives including methyl esters which contain a free or potentially free reducing group.

Procedure: The method of Dubois et al. [23] was used with wavelength at 470nm.

2.10 Collection of Blood Samples for Hemagglutination Assay

A 2ml of blood was collected from individuals of blood groups O^+, A^+ and B^+ for the hemagglutination assay. Plastic syringes with sterilized needles were used to collect the blood group O^+, A^+ and B^+ respectively from the vein dilations of the pupils concerned.

Procedure: One ml of the collected blood samples was added to 4 times its volume with normal saline and subsequently washed.

2.11 Preparation of Cell for Hemagglutination Test

Washing the red blood cells involved centrifuging its volume 3 times in normal saline at 4000 rpm for 10 mins. A drop of the washed red blood cell was placed on the slide and added a drop of the lectin. A cover slip was used to cover the prepared cells on the slide. It was viewed with the microscope using x40 objective (wet preparation). Finally, the lectin blood cell clumps were identified and snaped using the camera lens.

2.12 Study Design

Forty-five (45) male Wistar albino rats weighing between 110 and 130 g were procured from Chris Experimental Farm and Research Laboratory, Awka and randomized into nine (9) groups of five (5) rats each and used for the study. The rats were acclimatized for a period of one week before the commencement of the study. The grouping is as follows

Group A: Normal control, Group B: Wounded without treatment (pure petroleum jelly only), Group C: Wounded + std drug (3.6 mg/kg of amoxicillin) , Group D: Wounded + 2mg/kg bw lectin extract (oral), Group E: Wounded + 4 mg/kg bw lectin extract (oral), Group F: Wounded + 8 mg/kg bw lectin extract (oral), Group G: Wounded + 2mg/kg bw lectin extract (topical), Group H: Wounded +4mg/kg bw lectin extract (topical), Group I: Wounded + 8mg/kg bw lectin extract (topical).

2.13 Induction of Wound

The rats were anesthetized by intraperitoneal injection of xylazine hydrochloride (10mg/kg) and

ketamine hydrochloride (25mg/kg), and their dorsal surface hair was trimmed with an electric clipper. The dorsum of all rats was rinsed with a 10% povidone-iodine solution. Shaving stick was gently used on the hair removal area to inflict lesion/wound within a 20 mm circumference on the rats. After fully recovering from anesthesia, a photograph of the dorsum of the rat was taken from a 24cm standard height.

2.14 C-reactive Protein analysis using Karabag (2018) Method

Principle: The determination of C-reactive protein is carried out using an essential reagent required for an immune enzyme metric assay which include high affinity and specificity antibodies (enzyme and immobilized) with different and distinct epitope recognition in excess and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CRP antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labelled antibody and serum containing the native antigen reaction without competition or steric hindrance to form a soluble sandwich complex. The interaction is illustrated by the following equation.

enzAB (m) + AGcrp = AB(m) – Abm – Agcrp – AB(m)
AB(m) = Biotinylated monoclonal antibody (excess Quantity)
Agcrp = Native Antigrn (variable quantity)
AB(m) = Enzyme labelled antibody (excess quantity)
AB(p) Agcrp – AB(m) = antigen – Antibody sandwich complex
Ka = Rate constant of Association
K – a = Rate of constant of Dissociation.

Procedures: Before proceeding with assay. All reagents, serum references and control will be brought to a room at a temperature (20-27^{o}C).

i. The microplates well for each serum references control and sample specimen was formatted and assayed in duplicate. unusual microwell strips was replaced back into the aluminum bag seal and store at 2-80c
ii. A total amount of0.035ml (25 microliter) of the appropriate serum references was diluted control and patient's specimen.
iii. There was an addition of 0.100 (100microliter) of the CRP Enzyme reagents to each well. It is important to dispense all reagents close to the bottom of the well. (NB: A multichannel pipette is used quickly to dispense the enzyme reagents to avoid drifts if the dispensing is to take more than a few minutes.
iv. The microplate was swirled gently for 20-30 seconds to mix and cover.
v. It was incubated for 15 minutes at room temperature.
vi. The contents of the microplate were discarded by decantation or aspiration. If decanting tap and blot the plate dry with absorbent paper.
vii. There was an addition of 0.350ml (350 microliters) of wash buffer, decant (lap and blot) or aspirate. It will be repeated two (2) additional times for a total of three (3) washes.
viii. There was an addition of 0.100ml (100 microliters) of working substrate solution to all wells.

NB: Do not shake the plate after addition.

ix. It was incubated at room temperature for 15 minutes.
x. There was an addition of 0.050ml (50 microliters) of stop solution to each well and mix (gently) for 15-20 seconds.
xi. The absorbance was read in each well at 450nm (using a reference wavelength of 620 – 630 nm to minimize well imperfections) in a microplate reader. The result was read within thirty (30) minutes of adding stop solutions.

2.15 Statistical Analysis

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences software for windows version 25 (SPSS Inc., Chicago, Illinois, USA). All the data collected were expressed as Mean ± SEM. Statistical analysis of the results obtained were performed by using ANOVA Tests to determine if significant difference exists between the mean of the test and control groups. The limit of significance was set at $p<0.05$.

3. RESULTS

3.1 Hemagglutination Assay

Agglutination involves clumping of cells. Lectin protein enhances red blood cells clumping (hemagglutinins) as observed in Fig. 1a to 1c below. The control Group O +ve had free red blood cells without lectin (no clumping).

Table 1. Weight of *Pleurotus tuber-regium* sample

Sample	Weight (g)
Fresh weight	612.26
Dry weight	553.86

Table 2. Protein and carbohydrate content

Parameter	Mean ± SD
Protein	0.307±0.021 mg/ml
Glucose	49.55±4.56 mg/L
Sucrose	33.47±4.48 mg/L

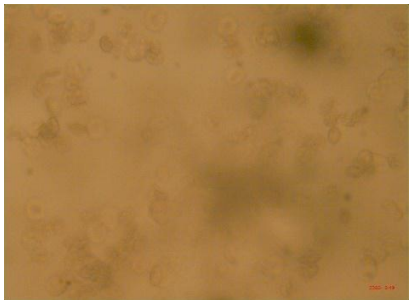

Fig. 1a. Group A +ve

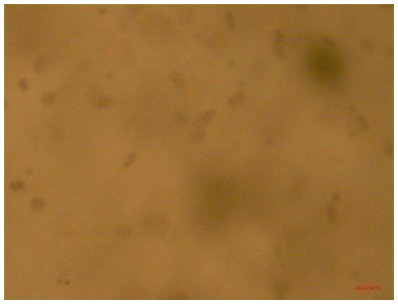

Fig. 1b. Group B +ve

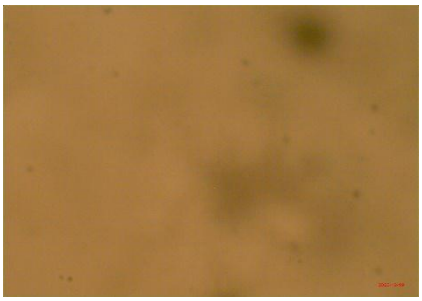

Fig. 1c. Group O +ve

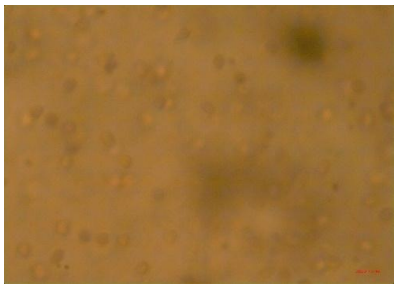

Fig. 1d. Group O control

3.2 Hematological Findings

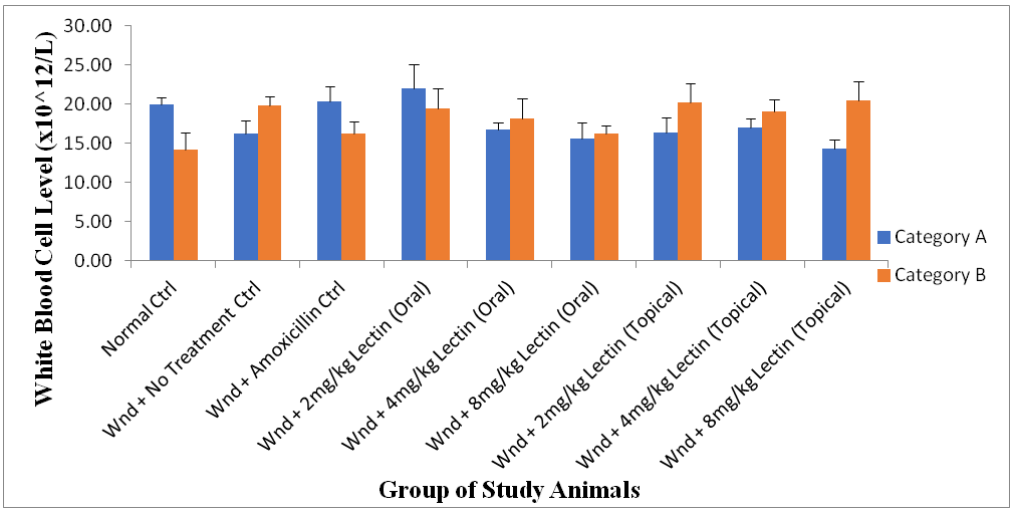

Fig. 2. White blood cell (WBC) count of the study animals

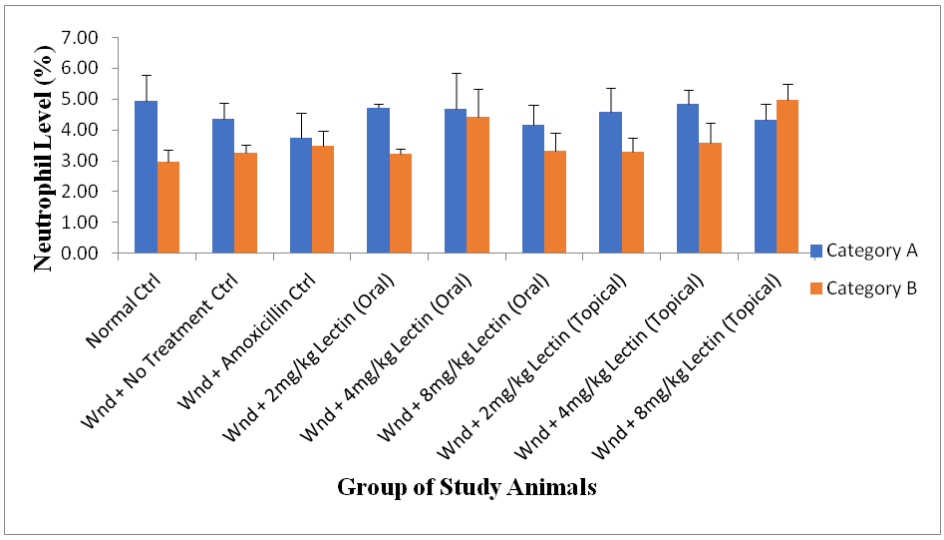

Fig. 3. Neutrophil levels of the study animals

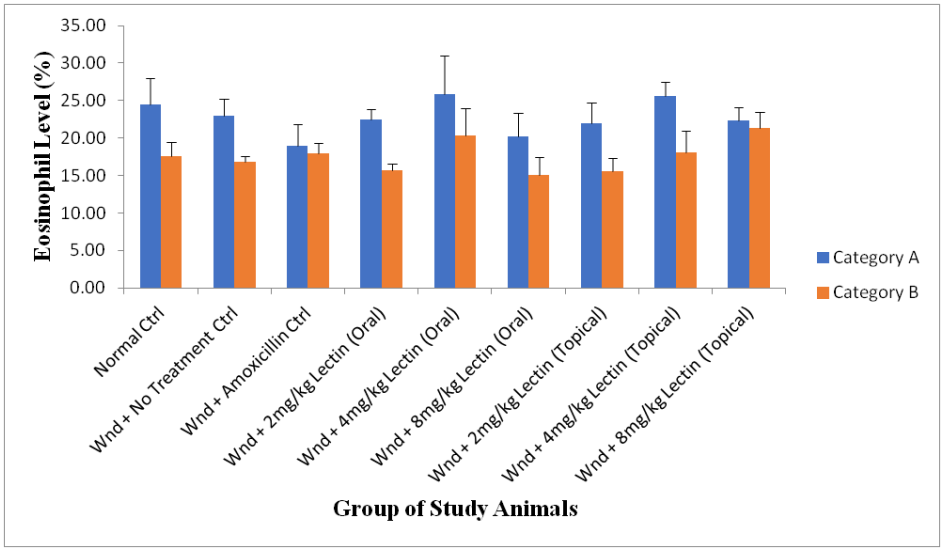

Fig. 4. Eosinophil levels of the study animals

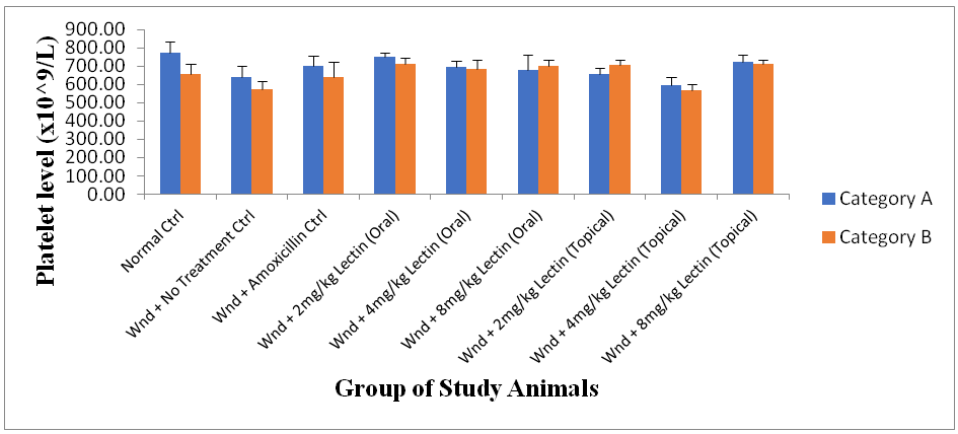

Fig. 5. Platelet levels of the study animals

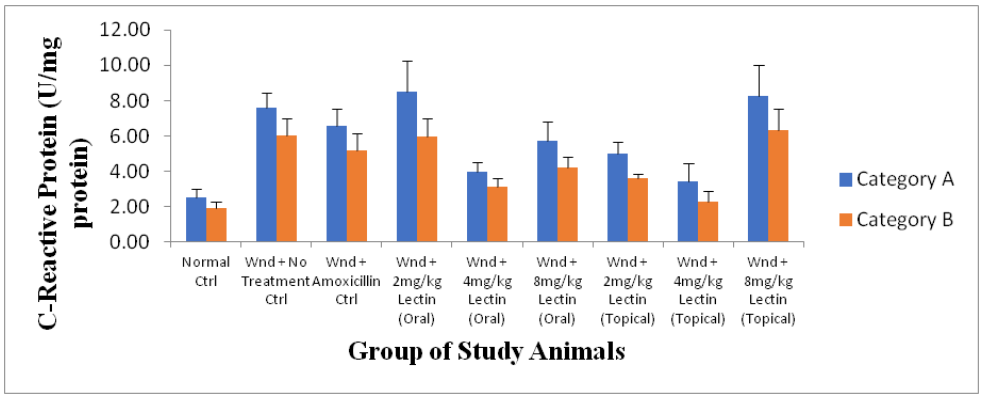

Fig. 6. C-Reactive protein levels of the study animals

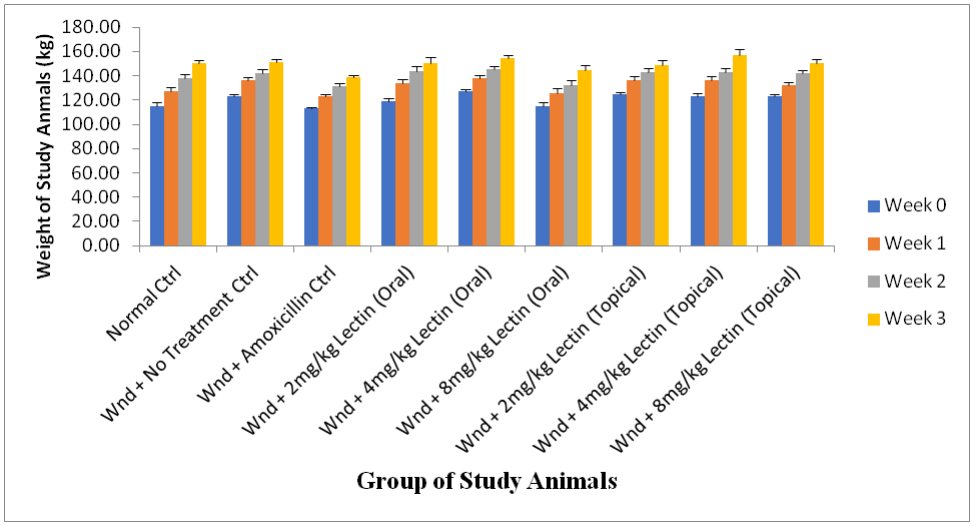

Fig. 7. Weight (kg) of the study animals

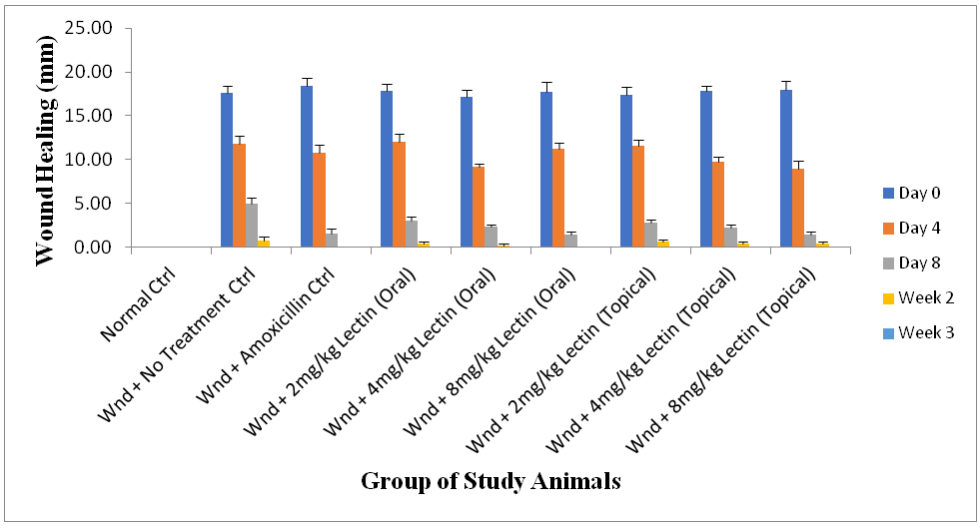

Fig. 8. Wound healing (mm) of the study animals

4. DISCUSSION

At normal control in Fig. 2, Category A group had a WBC value of 20.00 ± 0.87/L, while category B had 14.15 ± 2.16/L. The difference was non-significant, hence p>0.05. With no treatment, negative control, the WBC level was raised to 19.79 ± 1.22/L for the category B group. This was in line with WBC levels on wounding / inflammation typified by microphages and monocytes being attracted to the site of injury and peak at 48-72 hours after injury [24-26]. However, on category A group the WBC level dropped to 1624 ± 1.61. The difference was non-significant, p ≥ 0.05. The 36mg/kg amoxicillin application to the wounded rats on Category B group gave a decreased WBC level of 16.28 ± 1.46/L Amoxicillin cleared off all the bacteria by transpeptidation reaction [27]. The increased WBC level in category A group of 20.31 ± 1.98/L was non-significant, P ≥ 0.05. This implied that analysis in Category B group had stabilized to experimental processes of no treatment, and amoxicillin application than category A group. Hence, their differences were non-significant p ≥ 0.05. At 2kg/kg lectin oral administration, recorded the highest values on WBC levels of 21.98 ±1.09/L for Category A and 19.47±2.55/L for Category B group respectively. These high increases were due to the activation of the lectin pathway via the C1q of the classical pathway [28,29].

On topical administration of lectin to the wounded rats, showed very high WBC levels on category B compared to category A group, though these differences were non-significant, p ≥ 0.05. With 2mg/kg lection topical, category B group recorded 20.21 ± 2.48/L, while category A group had 16.38 ± 1.95/L. These high levels were due to factors as exposure of the wounds to the environment, vehicle of route of administration of lectin by using balms/oils (inert substances) and the lectin pathway application.

For normal control in Fig. 3, the Neutrophil level at category A group was 4.96 ± 0.82% compared to category B group with 2.96 ± 0.39%. At no treatment control, Category B group increased to 3.26 ± 0.25%. Neutrophils are the first responders as they are swiftly recruited, constituting about 50% of all cell at the injury site within 24 hours after injury [30]. The primary functions of neutrophils at the injury safe includes compacting invading pathogens via various antimicrobial responses; involving phagocytosis, toxic granules, oxidative burst and neutrophil extracellular traps (NETs) [24,31,32]. While at category A group, with no treatment control, the neutrophil level decreased slightly to 4.36 ± 0.52%. The difference between the normal control was not significant, p ≥ 0.05. On application 36mg/kg of amoxicillin to the wounded rats to category A group, there was a decrease in neutrophil, recorded as 3.76 ± 0.79%, implying that most of the bacteria were cleared by the amoxicillin, while a slight increase in neutrophils were recorded in group B; 3.48 ± 0.48%, indicating that complement alternate pathway was involved activated by dead bacterial cells and cell walls respectively [25].

On oral administration of lectin to wounded rats, there was a dose dependent decline for category A group with highest value at 2mg/kg lectin oral; with 4.72+0.14% to 8mg/kg lectin oral; with 4.18 ± 0.63%. While for category B group, showed a slight dose dependent increase from 2mg/1kg lectin oral to the highest value for 4mg/kg lectin with 4.42 + 0.92%. These increases in Neutrophil levels were attributed to the lectin pathway via the Clq classical pathway of the complement system [28]. On topical application of lectin to the wounded rats, there was a notable slight increase on dose dependent application for category A group. The highest report was with 4mg/kg lectin topical application, 4.84 ± 0.46%. while on category B group on topical application, notable increase on dose dependent was observed, the highest value reported at 8mg/kg lectin with 4.98 ± 0.52% neutrophil. These high increased values for neutrophils on topical application had same pattern in WBC level, topical application due to same reasons; exposure of the wound; vehicle of administration and the complement lectin activation factor.

Eosinophils less commonly called acidophils are one of the immune system components responsible for combating multicellular parasites and certain infections in vertebrates. They form about 2 to 3% of WBC and are responsible for tissue damage, inflammation, and allergy [25]. The eosinophil levels in category A showed higher values compared to category B group with p ≥ 0.05. At normal control in Fig. 4, category A group had value of 24.54 ± 3.46%, and category B group had 17.64+1.81%. Also with no treatment control, both category A and B groups had decline in numbers of eosinophils. Group A had 22.96 ± 2.32% while group B had 16.82 ± 0.78%, indicating that eosinophils were not attracted to the site of injuring and the differences were non-significant, p ≥ 0.05. On

36mg/kg amoxicillin application, both category A and B groups showed increases, with remarkable increase in category B of 17.92 ± 1.45% above its normal and no treatment control. The presence of dead bacterial cell wall activates the alternate pathway of complement system generating factors as C3b with receptors for eosinophils by chemotaxis [29]. The oral administration of lectin to the wounded rats, had a dose dependent increase in eosinophil in both category A and B groups; having the highest levels with 4mg/kg lectin oral, for category A 25.84 ± 5.22% while category B had 20.28 ± 3.70%. Hence the lectin pathway was activated through the Clq of the classical pathway generating C3b, C4 and including Histamine (H_1 & H_2) receptors on eosinophils by chemotaxis attraction [28,25].

On topical application of lectin to the wounded rats, there was also a dose dependent increase in both category A and B groups. For category A group, with 4mg/kg lectin tropical had the highest value of 25.64 ± 1.83% while with category B group 8mg/kg lectin topical had the highest values of 21.30 ± 2.17%. These high topical values were due to factors as exposure to the environment, vehicle of administration and the complement activation factor. The difference between the highest oral lectin administration in category A from 4mg/kg lectin value at 25.84 ± 5.22% from the normal control with 24.54 ± 3.46% was none significant $p \geq 0.05$. The difference in category B group from the highest topical at 8mg/kg/lectin value at 21.30 ± 2.17% to the normal control with 17.64+1.81% was significant; $p \leq 0.05$. Hence, in category B period, within the 28 days the analysis last, after the first 10 days of analysis, the rats within the category B period would probably be showing signs of allergic responses.

Platelets also called Thrombocytosis are small colourless cell fragments in the blood that form clots and stop or prevent bleeding [20]. Platelets have central role in innate immunity initiating and participating in multiple inflammatory processes, directly binding pathogens and even destroying them [21], formation of NETs [31], and in wound healing, [33]. At normal control in Fig. 5, the platelets levels were higher in Category A group; 770.60 ± 63.61 ug/L than in category B group; 654.80±58.84 ug/L. However, the differences were significance; $p \leq 0.05$; indicating within category A group the rats were not stable. A 2mg/kg oral lectin administration had values of 752.80±21.46ug/L for category A group and 712.00 ± 31.76ug/L for category B group. These highest values observed for 2mg/kg oral lectin administration was due to activation of the lectin pathway by C1q [28]. Both the oral lectin administration and the topical lectin application to the wounded ruts had highest platelets levels than the no treatment (negative control) 642.00±57.68 ug/L for Category A group and 573.80±44.69 ug/L for Category B group; except those administered with 4mg/kg topical with values for A group; 595.80 ± 43.70 ug/L and for B group; 569.60 ± 32.24ug/L. These results showed that lectin mobilized more platelets factors than the negative control. Hence the healing effect observed in the treated rate were as a result of the agglutinating/clumping of platelet factors by lectin, that served as means of attachment of different cell types of other cells including viruses via the surface carbohydrate of the lectins [33,34].

The C-reactive protein (CRP) levels showed the same pattern in both category A and B groups. As an acute phase protein with less than 20 hours half-life in the plasma, its normal concentration in category A group was 2.56 ± 0.46 ng/L, while in group B, was 1.94 ± 0.37 ng/L. The differences between the two groups were non-significant, hence $P \geq 0.05$. With no treatment (negative control) in Fig. 6. the CRP levels as a marker of inflammation rose to 3 time sits normal fold and in A group; 7.58 ± 0.55 ng/L and B group, 6.06 ± 0.96 ng/L [35]. Application of 36 mg/kg amoxicillin to the wounded rats showed a slight decrease in CRP levels that was non-significant $p \geq 0.05$. The A group had 6.56 ± 1.00 ng/L while the B group had 5.18 ± 0.99 ng/l. These values were due to activation of the alternate pathway of complement fixation via the dead bacteria cell-wall surfaced as substrates [29]. Application of 2mg/kg lectin oral administration had the highest CRP levels for Category A; 8.52 ± 1.76 ng/l and group B; 5.96 ± 1.02 ng/l. These high values were due to lectin activation by the C1q classical pathway [28]. The topical administration of lectin had highest value with 8 mg/kg topical, with 8.26 ± 1.76 mg/l for A group and 6.32 ± 1.24 mg/l for B group. These high CRP values for topical applications had same explanation for neutrophils topical application.

The rats in their respective groupings were weighted in kilograms (kg) from week zero to the third (3^{rd}) week. Normal control group in Fig. 7 had 115.00 ± 2.88kg on week zero, and gradually increased in weight to 150.60 ± 2.40 kg

on week 3; and gained 31.3% kg weight. Rats on no treatment (negative control) had a slight increase in weight of 23% from week zero to the 3rd week. These group involved self-healing processes of haemostasis, inflammation, proliferation/tissue remodelling and maturation processes [3,24]. The rats in these groups would be feeding maximally to maintain the self-healing process [7]. When 36 mg/kg amoxicillin was administered to the rats, from week zero to the 3rd week, there was a slight decrease in weight recorded as 22.4%. These decrease in weight compared to the normal and no treatment control was less than or equal to significant $P \leq 0.05$. The amoxicillin, an aminopenicillin acts by transpeptidation reaction [27] and at higher concentrations, was toxic to the cells and tissues of the rats and was not nutritional [36]. Oral administration of lectin to the rats showed a slight increase in weight from 2 mg/kg oral with 26.2% to 26.4% weight increase for 8 mg/kg oral lectin application. Lectin topical administration had its highest weight increase in 4 mg/kg topical with 27.3%. The lectin oral administration showed it was highly nutritional and had little or no inhibitory effect on the growth of the rats and with unique national products [37].

On normal control groups in Fig. 8, member rats had no wound cut in them (mm), therefore at Day O to week 3, maintains no healing process, recorded as 0.00 ± 0.00. With no treatment (-ve control) groups had 17.60 ± 0.31 mm wound cut on Day O, which gradually reduced to 0.80 ± 0.32 mm on week 2. Complete healing was on week 3 with 0.00 ± 0.00 mm, Rats in these groups were healed by undergoing the four processes of healing [37]; and with the available rat feeds provided [7].

With 36 mg/kg amoxicillin application, rats in this group had 18.40 ± 0.87 mm wound cut, and it took 2 week for complete healing, 0.00 ± 0.00 mm. However, the drug was not nutritional and toxic to the cells/tissues of the rats at cumulative concentrations [38]. Oral administration of lectin to the wounded rats (mm) reduced the diameter of healing at 0.40 ± 0.24 mm at 2 weeks for 2 mg/kg lectin to 0.20 ± 0.20 for 2 weeks at 4mg/kg lectin and to 0.00 ± 0.00 for 2 weeks at 8 mg/kg lectin application. Hence, 8 mg/kg lectin oral administration completely healed the wound for 2 weeks period, the same period amoxicillin used to heal the wound. However, oral administration of lectin was nutritional and added weight to the rats as noted on the weight column [7,3,36]; Topical application of the lectin extracts on the wounds (mm) had healing affects. Though complete healing was recorded in week 3 with 0.00 ± 0.00 [39,40].

5. CONCLUSION

The lectin extract from edible mushroom *Pleurotus tuber-regium* clinically showed its healing effects on the acute wound induced on male albino rats. The lectin application to the wounded rats enhanced neutrophil migration to the site of injury, platelets aggregation for wound healing, fastens clumping (agglutinins) of human red blood cells and hastens inflammatory responses of C-reactive protein. The amoxicillin application to the wounded rats triggered the alternate pathways of the complement system, while both the oral and topical applications triggered the Lectin pathway of the complement system.

Rats in category B group after the first 10 days into the study, would probably be showing signs of allergic responses. Amoxicillin application showed a decrease in weight of 22.4% while both oral and topical administration of lectin had increase in bodyweight from 26.4% to 27.3%.

Lectin oral administration at 8 mg/kg and 36 mg/kg amoxicillin application had two weeks for complete healing of the induced wound. However, oral administration of lectin was nutritional thereby leading to increased body weight observed.

ETHICAL APPROVAL

This research work was approved and supervised by Nnamdi Azikiwe University-Animal Research Ethics Committee (NAU-AREC) in accordance with Animal Care and Use in Research, Education and Testing (ACURET). The ethical approval number as issued by the Nnamdi Azikiwe University-Animal Research Ethics Committee is NAU/AREC/2023/00003.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Reid T, Fore F, Chidzwondo F, Kashangura C, Stray B, Mduluza F. Screening of mushrooms from the woodland of Zimbabwe: Occurrence of lectins and partial purification of a mucin specific lectin from Boletus edulis. Plos one. 2022;17(4)e O265494.
2. Rarn SS, Hemant PK, Jagat RK. Mushroom lectins as promising anticancer substances. 2016;17(8):797-807.
3. Santos AFS, Da Silva, MDC, Napoleão TH, Paiva PMG, Correia MTS, Coelho LC. Lectins: Function, structure, biological properties and potential applications. Current Topics in Peptide & Protein Research. 2014;15: 41-62.
4. Katoch R, Tripathi A. Research advances and prospects of legume lectins. J Bio Sci. 46(4);104.
5. Deadlock DM; Bartus HF, Zajac J, Actor P. Analysis of parameters affecting the hemagglutination activity of Escherichia Coli possessing colonization factor antigens; improved medium for observing erythrocyte agglutination. J Clin Microbiol. 1981;2021;(2):301-8.
6. Yousra AE, E SE, Marwa MA, Moua HH. A Comprehensive Insight into Fungal Enzymes: Structure, Classification and Their Role in Mankind's Challenges. J Fungi (Basel). 2021;8(1): 23.
7. Bell V, Silva CRPG, Guina J, Fernandes TH. Mushrooms as future generation of healthy foods. Front Nutri. 2022;9: 1050099.
8. Diaz EM, Vicente-Manzanare M, Sacristan M, Vicente C, Legaz ME. Fungal lectin of *Peltigera canina* induces chemotropism of compatible nostoc cells by constriction-relaxation pulses of cyanobiont cytoskeleton. Plant Signalling & Behaviour. 2011;6:1525–1536.
9. Jayaraman J. Laboratory Manual in Biochemistry. Wiley Eastern limited publishers, New Delhi, Bangalore, Bombay, Calcutta. 1992;82-83.
10. Allen PC, Hill EA, and Stokes AM. Plasma proteins. Analytical and preparative techniques. Blackwell Scientific Publications Oxford London. 1997; 113-130.
11. Ukoha, AI. Gel filtration methods. In techniques in clinical Immunology; ed C.A. Thompson (Oxford, London, Boston: Blackwell Scientific Publication).1998;136.
12. Lucy CA. "Evolution of ion-exchange from Moses to the Marahatha project to Modern Times" Journal of Chromatography 1000. 2003;(1-2):711-24.
13. Guillamón E, García-Lafuente A, Lozano M., D'Arrigo M., Rostagno MA, Villares A, Martínez JA. Edible mushrooms: Role in the prevention of cardiovascular diseases. Fitoterapia. 2010;81:715–723.
14. Mohamed AAH, Razina R, Evelin T, Torn WM, and Joe T. Mushroom Lectins: Specificity, Structure and bioactivity relevant to human disease. Int J Mol Sci. 2015;16(4):7802-38.
15. Stavitsky AB. Agglutination (2nd Ed.). Encyclopaedia of Immunology. 1998; 56-59.
16. Jacobs L, Nawrot TS, De Geus B, Meeusen R, Degraeuwe B, Bernard A, Sughis M, Nemery B, Panis LI. Subclinical responses in healthy cyclists briefly exposed to traffic-related air pollution: an intervention study. Environmental Health. 2010;9(64):64.26.
17. Waugh DJ, Wilson C. The interleukin-8 pathway in cancer. Clinical Cancer Research. 2008;14 (21):6735–41.
18. Tak T, Tesselaar K, Pillay J, Borghans JA, Koenderman, L. "What's your age again? Determination of human neutrophil half-lives revisited". Journal of Leukocyte Biology 2013; 94 (4): 595–601.
19. Wheater PR, Stevens A. Wheater's basic histopathology: A colour atlas and text. Edinburgh: Churchill Livingston; 2002.
20. Laki K. Our ancient heritage in blood clotting and some of its consequences. Annals of the New York Academy of Sciences. 1972;202(1):297–307.
21. Kubes P ,Jonathan CL, Jonathan S, Braedon M, Stephen G, Simon C, Betty G, France B, Lorenzo F. Neutrophil extracellular traps sequester circulating tumor cells and promote metastasis. The Journal of Clinical Investigation; 2013. DOI:10.1172/JCI67484.
22. Lowry OH, Rosebrough NJ, Farr Al, Randall RJ. Protein measurement with the Folin phenol reagent. J. Biol Chem. 1951;193:265-267.

23. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 1956;18: 350-356.
24. Amro MS, Daniel RB. Acute Inflammation in Tissue Healing. Int J Mol Sci. 2023;24(1);641.
25. Salimonu LS. Basic Immunology for Students of Medicine and Biology. College Press and Publishers Limited, Jerico GRA Ibadan. 2004;48-54.
26. Yáñez A, Simon GC, Andre O, David EM, Benjamin PB, Dennis JH, Nathan S, H. Leighton Grimes, Helen SG. Granulocyte-monocyte progenitors and monocyte-dendritic cell progenitors independently produce functionally distinct monocytes. Immunity. 2017;47(5):890–902.e4.
27. Enzyme Structure Data Base. EC 3 4 16 .4 serin type D Ala- D-Ala Carboxypeptidase. Retrieved from Archieve; 2008.
28. Thompson D, Pepys MB, Wood SP. The physiological structure of human C-reactive protein and its complex with phosphocholine. Structure. 1999;7(2): 169–77.
29. Abbas AK, Lichtman AH. Pathways of Complement Activation en basic immunology; Functions and Disorders of the Immune system, 3rd Edition, Saunders Elsevier publishers. 2006;159-165.
30. De Oliveriva S, Rosoveski E, Itutteniocher A. Neutrophil migration in Infection and wound Repair; Going forward in Reverse. Nat Rev Immunol. 2016;16; 378-391.
31. Shuainan Z, Ying Y, Yun R, Living Y, Huilin W, Xiaomm L, Lin J, Yan H, Hao Z, Changhong M, Kefang Guo.The emerging roles of Neutrophil extracellular traps in wound healing. Cell Death Dis. 2021;12(11):984.
32. Brinkman V, Rekchard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychnnsky A. Neutrophil Extracellular Traps Kill Bacteria. Science. 2004;303;1532-1535.
33. Alan TN, Paquita N, Mikel S, Isabel A, Eduardo A. Platelet and Wound Healing. Frontiers in Bioscience. 2004;13:3525-3548.
34. Florian G, Zerkah A, Gerhild R, Shuxia F, Leo N. Migrating Platelets Are Mechano-Scavengers that collect and Bundle Bacteria. 2017;171(6):1368-1382.
35. Helena E, Jesper k, Heu-Yun L, Yi Wu, Irving K, Jonus W, and Christopher S. The complex Role of C-Reactive Protein in Systemic Lupus Erythematosus. J Clin Med. 2022;(24);5837.
36. Carmelia M, Bethy S. The relationship between nutrition and the immune system. Front. Nutr. 2022;9;1082500.
37. Marilyn SC, Laura AC, Nayely LL, Erick PG, Luis AJO, J Basillo H. Wound Healing Properties of Natural Products: Mechanisms of Action. Molecules. 2023;28(2): 598.
38. Hina N, Fletcher D, Poindessous-Jazat F, Martinez V. Hyperalgesia induced by low-dose opioid treatment before orthopaedic surgery. European Journal of Anaesthesiology. 2015; 32(4): 255-261.
39. Gillitzer R, Goebeler M. Chemokines in cutaneous wound healing. J Leukoc Biol. 2001;69(4):513-21.
40. Stefania V, Sara C, Martina P, Giovanna DE, Carla T, Fernanda A, Anna Maria DA. Phytochemistry and biological activity of medicinal plants in wound healing: An overview of current research. Molecules. 2022;27(11):3566.

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