EFFECTS OF PARKIA BIGLOBOSA LEAF EXTRACT ON SURGICAL WOUND HEALING IN ALBINO RAT

BY

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FACULTY OF LIFE SCIENCES

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DECEMBER, 2022

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IN PARTIAL FULFILLMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) HONOURS IN ANIMAL AND ENVIRONMENTAL BIOLOGY

DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY

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EDO STATE

DECEMBER, 2022.

CERTIFICATION

This is to certify that this undergraduate project was carried out by **MATHEW BLESSING EWERE** with the matriculation number **LSC1704939** in the Department of Animal and Environmental Biology, Faculty Of Life Sciences University of Benin, Benin City.

……………………………………… 20th December 2022 ………………………..……………..

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………………………………....... ……………………….…..…………

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…………………………………… ……………………………………

External supervisor Date

DEDICATION

This project is dedicated to God Almighty for giving me the knowledge and guidance to carry out this work. Also to my beloved parents and family for their moral and financial support throughout my stay at the university and especially during my project work.

ACKNOWLEDGEMENTS

I want to give thanks to God, the Almighty, who is my creator and is responsible for my life, my health, and his eternal love.

I would like to thank my parents, Mr. & Mrs. Mathew Osunde, for their financial assistance, guidance, and moral support. I also want to thank my devoted husband, Mr. Kingsley Okafor, and my amazing siblings, Chinyere, Jeremiah, Richard, and Racheal, for all of their love and support.

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Finally, I would like to thank the Animal and Environmental Biology department at the University of Benin for giving me the chance to put what I have learned into practice.

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ABSTRACT

This study was carried out to determine the wound healing potency of *Parkia biglobosa* ethanol leaf extract on surgical wounds using animal model. Thirty-two (32) healthy female albino rats (*Rattus norvegicus*), two months old, with an average weight of about 188g were purchased. Normal excision wounded animals in Group 1 (Control) were only given food and water. Group 2 (Standard drug) were given cicatrin powder to treat their wounds. Group 3 were given 10% *Parkia biglobosa* extract to treat their wounds. Group 4 were given 20% *Parkia biglobosa* extract to treat their wounds. After seven days, serum blood samples were taken, analyzed for anti-inflammatory. The results of showed that the extracts of *P. biglobosa* had positive effect on wounded healing. Tumour necrosis factor (TNF-α) of group 1 animals range from 136.0 pg/ml to 110.5 pg/ml, group 2 animals range from 87.0 pg/ml to 117.6 pg/ml, group 3 animals range from 123.45 pg/ml to 117.6 pg/ml, while group 4 animals range from 116.7 pg/ml to 133.6 pg/ml. The Superoxide dismutase (SOD) values of the treated animals were as follows: group 1 animals range from 12.6 U/mg to 18.95 U/mg, group 2 animals range from 17.85 U/mg to 25.15 U/mg, group 3 animals range from 14.2 U/mg to 23.9 U/mg, while group 4 animals range from 14.8 U/mg to 16.1 U/mg. The study recommends that the potential and safety of this plant extract, as well as their possible protective mechanisms, should be determined before administration into humans.

CHAPTER ONE

1.0 INTRODUCTION

The African locust bean tree, *Parkia biglobosa* (Jacq.) R. Br. ex G. Don, is a perennial tree legume that is a member of the Fabaceae family and Mimosodeae subfamily. This study's objective is to find out how *Parkia biglobosa* ethanol leaf extract treats surgical wounds in albino rats (Abdullahi *et al.,* 2015). Any deterioration of the structural integrity of biological tissue, such as the skin, mucous membranes, and organ tissues, is referred to as a wound. Clean, clean-contaminated, contaminated, and dirty-infected wounds are all categorized as such. The bark of *Pakia biglobosa* is used as traditional medicine by the Hausa and Fulani people of Nigeria to cure wounds by applying the finely powdered bark to the open wounds. The extract can be made into an emulgel, which has more advantages than the powdered version (Soetan *et al.,* 2011). A very significant multifunctional tree called *Parkia biglobosa* is primarily used for food, medicine, and magico-therapeutic uses. The species of *P. biglobosa* is abundant in saponins, tannins, flavonoids, resins, carbohydrates, terpenoids, phenols, sterols, and cardiac glycosides, according to the aqueous extracts of the plant. *Parkia biglobosa's* physicochemical characteristics have been linked to both its therapeutic and nutritional advantages. Antimalarial, anti-helminthic, antibacterial, antidiabetic, antihypertensive, anti-inflammatory, analgesic, anti-carcinogenic, anti-trypanosomic, and antioxidant characteristics were demonstrated by *Parkia biglobosa* in pharmacological research (Ong *et al.,* 2011).

1.1 WOUND

The anatomical and cellular continuity of tissue is disrupted by wounds, which are physical injuries brought on by chemical, physical, thermal, microbiological, or immunological insult to the tissue (Ong *et al.,* 2012. Any deterioration of biological tissue's integrity, such as that of the skin, mucous membranes, or organ tissues, is referred to as a wound. These can result

from a variety of traumas, and it's crucial to make sure that wounds are cleaned and properly dressed to prevent the development of infection and more harm.

There are four categories into which wounds can be placed:

Class 1 scratches are considered clean. They are not infected, not inflamed, and mostly closed. If drainage of these wounds is required, a closed drainage method is required. Also, these wounds do not enter the respiratory, digestive, reproductive, or urinary tracts (Builders, 2012).

Class 2 wounds are considered clean and contaminated. These wounds show no unusual contamination. These ulcers enter the respiratory, digestive, genital, or urinary tracts. However, these wounds entered these pathways under controlled conditions (Adaramola *et al.,* 2012).

Class 3 wounds are taken into consideration to be consstaminated. These are fresh, open wounds which could end result from insult to sterile strategies or leakage from the gastrointestinal tract into the wound. Additionally, incisions made that bring about acute or loss of purulent infection are taken into consideration elegance three wounds (Henry *et al.,* 2013).

Class 4 wounds are taken into consideration to be dirty-infected. These wounds commonly end result from improperly cared for annoying wounds. Class four wounds show devitalized tissue, and that they maximum usually end result from microorganisms found in perforated viscera or the operative field.

1.2 WOUND HEALING

- The wound healing process consists of integrated cellular and biochemical cascades that lead to restoration of the structural and functional integrity of injured tissue. Repair of damaged tissue occurs as a sequence of events including (Sani, 2014):

- Hemostasis (blood clotting): withinside the first short time of harm acquired, platelets withinside the blood start to collect and keep on with the injured site. They alternate into an amorphous shape, extra appropriate for clotting, and that they launch chemical indicators to sell clotting. This effects withinside the activation of fibrin, which bureaucracy a mesh and acts as "glue" to bind platelets to every other. This makes a clot that serves to plug the destroy withinside the blood vessel, slowing/stopping in addition bleeding (Salam *et al.,* 2014).

- Inflammation: During this phase, broken and lifeless cells are cleared out, at the side of micro organism and different pathogens or particles. This occurs thru the system of phagocytosis, in which white blood cells engulf particles and break it. Platelet-derived increase elements are launched into the wound that reasons the migration and department of cells in the course of the proliferative phase (Iyamu *et al.,* 2014).

- Proliferation (new tissue growth): At this stage, angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction occur (Enoch and Price, 2004). During angiogenesis, vascular endothelial cells form new blood vessels (Chang et al., 2004). During fibrosis and granulation tissue formation, fibroblasts grow and form a new makeshift extracellular matrix by secreting collagen and fibronectin (Builders *et al.,* 2014). At the same time, re-epithelialization of the epidermis occurs, with epithelial cells proliferating and “crawling” over the wound bed to cover new tissue. During wound contraction, myofibroblasts reduce wound size by using mechanisms similar to smooth muscle cells to grasp and contract the wound edges. When a cell's work is nearly complete, unwanted cells undergo apoptosis (Singh *et al.,* 2015).

- Remodeling: During maturation and remodeling, collagen is reorganized along stress lines and unwanted cells are removed by programmed cell death.

1.3 ALBINO RATS

The albino rat *Rattus norvegicus* became advanced on the Wistar Institute in 1906 to be used in organic and clinical studies. Albino rats are one of the maximum often used laboratory animals withinside the world. Therefore, the albino rat have become synonymous with "laboratory rat." Although the term "albino rat" is usually used at present, the rat has been known as white rat, Daikoku rat, and ratte withinside the past. However, the foundation of albino laboratory rats stays unclear (Srisawat *et al.,* 2016).

The morphological functions of the rats consist of a huge head, lengthy ears, and tail duration this is usually much less than its frame duration. The first rat colony in America used for vitamins studies became began out in January 1908 through Elmer McCollum, after which the nutritive necessities of rats have been utilized by Thomas Burr Osborne and Lafayette Mendel to decide the information of protein vitamins. Laboratory rats are often situation to dissection or microdialysis to observe inner results on organs and the brain, which includes for most cancers or pharmacological studies (Ezema *et al.,* 2016). Laboratory rats now no longer sacrificed can be euthanized or, in a few cases, emerge as pets. Domestic rats range from wild rats in lots of ways (they may be calmer and considerably much less probable to bite, they are able to tolerate extra crowding, they breed in advance and convey extra offspring, and their brains, livers, kidneys, adrenal glands, and hearts are smaller). Scientists have bred many traces or "lines" of rats particularly for experimentation. Most are derived from the albino Wistar rat, which remains extensively used (Boye *et al.,* 2016). Other not unusualplace traces are the Sprague Dawley, Fischer 344, Holtzman albino traces, Long–Evans, and Lister black hooded rats. Inbred traces also are to be had however aren't as usually used as inbred mice.

1.4 AIMS AND OBJECTIVES

The aim of this study is to determine the wound-healing effects of ethanol leaf extract of *Parkia biglobosa* on surgical wounds in Albino rat.

Objectives of the experiment include

- To determine the healing rates of the rats after *Parkia biglobosa* has been administered to them.
- The efficacy of 2 concentrations of the extract on a known wound-healing drug

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 EFFECTS PARKIA BIGLOBOSA ON WOUND HEALING IN RATS

Ifesan *et al.* (2017) conducted an experiment on the efficacy of his *Parkia biglobosa* stem bark in treating burns. During the course of experiments, it was discovered that finely powdered stem bark of *P. biglobosa* exhibited potent wound-healing activity, and emulgel formulations enhanced wound-healing activity. Experiments were conducted by mixing P. stem bark powder and extract of *P. biglobosa* administered 1% sulfadiazine silver cream, also known as Dermazin®, to Wistar albino rats. The animals were burned and divided into 6 groups of 4 animals per group and designated A through F. Group E was treated with Dermazine® as a positive control, while Group F was treated as a negative control and thus was not treated with any substance. Another group was topically treated with emulgel containing powdered stem bark and *P. biglobosa* extract as follows. w), group D (5 wt%). At the end of the experiment, group C, who received emulgel containing 2.5% by weight of the herbal extract, showed superior burn healing compared to finely powdered stem bark. One wound showed a healing effect comparable to that of Dermazin®. Experimental results support the common use of *P. biglobosa* trunk bark to treat burns (Singh, 2019).

Builders *et al.* (2019) while conducting experiments on the effect of *Parkia biglobosa* extract on dexamethasone-induced hyperglycemia in rats and open skin wound healing in histological evaluation. During this experiment, 18 rats were given three different doses of *P. biglobosa* extract (25, 50, and 100 mg/kg body weight, orally), designated P1, P2, and P3, and 6 rats were Ketoconazole (24 mg/kg body weight) was administered. , p.o.) and referred to 14 consecutive days of open wound healing as P4 before daily administration of a prestandardized dose of dexamethasone (1 mg/kg body weight, intramuscularly) group 1. In

group 2, 18 rats were treated with 3 different doses of *P. biglobosa* extract (25, 50, and 100 mg/kg body weight, po), designated P5, P6, P7, and 6 rats received ketoconazole (24 mg/kg body weight). kg body weight). wt., p.o.) (Abioye *et al.,* 2013) and he is designated P8 for 14 consecutive days in open wound healing after dexamethasone-induced hyperglycemia (SáSantos *et al.,* 2018). Simultaneously, 6 normoglycemic rats were treated with an equal volume (0.2 ml saline) and designated P9. At the end of the experiment, the percent reduction in serum glucose concentration in dexamethasone-induced hyperglycemic animals before and after treatment was greater in the groups pretreated with 50 and 100 mg/kg *P. biglobosa* (14.9 and 19.21%, respectively) compared to ketoconazole which was only 16.5%. In the post-treatment group, the reduction of *P. biglobosa* at 100 mg/kg (17.7%) was greater than that of ketoconazole, which was only 16.6%. After histological evaluation, animals in the pretreatment group showed higher rating scales and improved healing compared to the posttreatment group. Therefore, the wound healing process was reduced in the dose-dependent pretreatment group. However not much work have been done on the effect of *Parkia biglobosa* in the treatment of surgical wounds in albino rats but similar works have been done on the effects of *Parkia biglobosa* extract on rats (Sheikh *et al.,* 2016).

Rathi *et al.* (2017) conducted an experiment on the effects of a methanol extract from his *Parkia biglobosa* stem bark on liver and kidney function in albino rats. The purpose of this experiment was to determine the beneficial properties of *Parkia biglobosa* as a medicine and its effects on vital organs in the body. At the end of the experiment, we found that the extract did not cause significant changes in some of the hepatic and haematological parameters, while showing a decrease in alkaline phosphatase activity and PCV concentration. The functional properties and effects of a methanolic extract of *Parkia virobosa* stem bark on liver and kidney function in rats could be determined after brief treatment with the extract.

The kidneys remained intact, reflecting the changes, but remained intact (Ogunyinka *et al.,* 2017).

Mondal *et al.* (2013) conducted an experiment on toxicity studies of *Parkia biglobosa* stem bark extract in rats. During the course of the experiment, kidneys and livers of animals treated with P. biglobosa aqueous extract for 14 days showed histopathological signs of pathological lesions. At the end of the study, the toxicity characteristics of methanol and water extracts of *P. biglobosa* stem bark in short-term exposure to the extracts, and the diversity of toxicity and chemical constituents of his *P. biglobosa* stem bark. *P. biglobosa* associated with the extraction solvent was discovered (Awotedu *et al.,* 2018).

Kuma *et al.* (2022) conducted a study on the wound healing effects of *Catharanthus roseus* extract in Sprague-Dawley rats. Madagascar periwinkle has been used to treat a variety of ailments, including diabetes. The healing ability of the extracts was evaluated based on wound contraction rate, epithelialization time, tensile strength (skin breaking strength), granulation tissue weight, and hydroxyproline content. The antibacterial activity of the flower extract against four microorganisms was also evaluated. *C. roseus* extract significantly increased wound fracture strength in the laceration model compared to controls ($P < 0.001$). Extract-treated wounds were epithelialized faster compared to control wounds, wound contraction rate was significantly increased ($P < 0.001$), wet and dry granulation tissue weights, and hydroxyproline content found to be significantly increased in the cavity wound model ($p < 0.05$). At the end of the study, increased wound contraction and tensile strength, increased hydroxyproline content, and antibacterial activity supported the use of *C. Roseus* in the topical management of wound healing (Heymann *et al.,* 2012).

Fayinminnu *et al.* (2017) carried out a research on the evaluation of the In Vivo healing activity of *Bacopa monniera* on different wound model in rats. 50% ethanol extract of dried

whole plant of *Bacopa monniera* was studied on wound models in rats. *Bacopa monniera* (25 mg/kg) was administered orally, once daily for 10 days incision and dead space wound models *Bacopa monniera* showed antimicrobial activity against skin pathogens, enhanced WBS, rate of contraction, skin collagen tissue formation, and early epithelization period with low scar area indicating enhanced healing. Healing effect was further substantiated by decreased free radicals and myeloperoxidase and enhanced antioxidants and connective tissue markers with histological evidence of more collagen formation in skin and deeper connective tissues.

Costa *et al.* (2013) carried out a research on the wound healing activity of *Cestrum nocturnum* leaves in wistar albino rats, ethanolic extract of *Cestrum nocturnum* (L.) leaves using excision and incision wound model whereby The wound healing effect was assessed by percentage wound contraction, epithelialization period, and histoarchitecture studies in excision wound model while breaking strength and hydroxyproline content in the incision wound model. This resulted in ointments containing different concentrations of *Cestrum nocturum* ethanolic extract (2% and 5% w/w) that significantly promoted wound healing activity in both models studied. Higher rate of wound contraction, shorter epithelialization time, higher skin breaking strength, and increased hydroxyproline content compared with animals in control and negative control groups treated with ethanolic extract of nightworm ointment observed in animals. A histopathological study of a group treated with an ethanolic extract from *Cestrum nocturnum* ointment also demonstrated efficacy in improving wound healing. From this, it was concluded that the ethanol extract from leaves of *Cestrum nocturnum* has a concentration-dependent wound-healing effect (Builders *et al.,* 2021).

Chhikara *et al.* (2018) conducted a study on the wound healing ability of licorice extract in a rat model. Licorice has antioxidant and anti-inflammatory properties, enhancing its valuable effects as a herbal medicine. In this study, through immunological, antioxidant,

histopathological, immunohistochemical and molecular studies, we investigated the potential of wound healing and the potential of alcoholic licorice extract to modulate cutaneous wound healing. A mechanism was investigated. Twenty-four Wistar rats were divided into three groups: a control group, a topical and an oral feeding group. Administration of licorice extract significantly increased total and differential white blood cell counts, neutrophil phagocytic activity, antioxidant biomarkers such as superoxide dismutase (SOD), glutathione peroxidase activity (GPx), and glutathione (GSH).) reduces levels of the oxidative stress marker malondialdehyde (MDA). Furthermore, histopathological findings showed complete re-epithelialization with increased collagen synthesis, whereas IHC results showed that α-SMA, PDGFR-α, FGFR1, and showed a significant increase in cytokeratin 14 expression. Licorice extract supplementation promoted wound healing by increasing angiogenesis and collagen deposition through upregulation of bFGF, VEGF, and TGF-β gene expression levels compared to the control group. UPLC-PDA-MS/MS helped authenticate the studied licorice species and identified 101 potential constituents that may be involved in licorice potential. Licorice has improved skin wound healing due to its free radical scavenging ability, strong antioxidant and anti-inflammatory effects. It can be used as a potential alternative therapy (Chanu *et al.,* 2018).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 EXPERIMENTAL DESIGN

3.1.1 Experimental Animals

From the breeding facility of the Department of Anatomy, Faculty of Basic and Medical Sciences, University of Benin, Benin City, 32 healthy female albino rats, *Rattus norvegicus,* two months old, with an average weight of about 188g were purchased. For a week, they were housed in plastic cages with wire mesh coverings and fed rat pellets supplied from the animal house of the department of anatomy at the University of Benin in Benin City. Water was available at all times. The bedding for the cages was made of wheat straws.

3.1.2 Treatment Protocol

Three of the four animal groups received treatment, but one group received simply food and water for 21 days. For a total of 21 days, all preparations were applied topically once each day as follows: Normal excision wounded animals in Group 1 (Control) were only given food and water. Group 2 (Standard drug) were given cicatrin powder to treat their wounds. Group 3 (Test 1) were given 10% *Parkia biglobosa* extract to treat their wounds. Group 4 (Test 2) were given 20% *Parkia biglobosa* extract to treat their wounds. After seven days, samples of the necessary tissues and blood were taken, and they were analyzed for histological alterations, haematological changes, and the overall effects of *Parkia biglobosa* extract on albino rat wound healing.

3.2 PREPARATION OF *PARKIA BIGLOBOSA* (JACQ.) BENTH EXTRACT

Parkia biglobosa leaf cleaned with sterile water, allowed to air dry for 10 days, and then was reduced to semi-powdered particles by being macerated using a mortar and pestle. The leaf was steeped for three days in 400mL of ethanol and acetone with 100g of each. Then, man

filter paper was used to filter it. The filtrate was dried using a rotary evaporator, and crude extracts of ethanol and acetone were produced for antimicrobial testing.

3.3 EXCISION WOUND MODEL

According to Panchatcharam, this surgical treatment was carried out. The animals' backs were shaved, and the wounding procedures were performed while they were under the anesthesia of thiopentone sodium (40 mg/kg, ip). Excision wounds with an average diameter of 2.5 cm were created after at least 5 mm of space had been left around the ears, and they were treated topically each day until the day of epithelization (Figure 1). The physical characteristics of wound healing, such as scar characteristics, epithelization, and wound closure (contraction), were noted. The wound contraction was measured as a percentage reduction of the initial wound area by tracing 1 mm2 graph paper on the day of wounding and then on alternate days until healing was complete (Morton & Malone, 1997).

3.4 COLLECTION OF SAMPLES FOR HAEMATOLOGY

Using 5ml disposable syringes, blood samples were drawn from the dorsal aorta of dissected albino rats' hearts and put into sterile vials containing the anticoagulant lithium heparin. The samples were centrifuged shortly after collection, and the serum was then put into simple bottles. The samples were maintained on ice before being sent to the Zone 6 Diagnostic Laboratory's Haematological Unit in Calabar, Cross River State, Nigeria. There, they were examined for biomarkers of oxidative stress, such as tumor necrosis factor alpha (TNF alpha) and superoxide dismutase (SOD).

3.5 PRO-INFLAMMATORY BIOMAKERS

3.5.1 Tumor necrosis factor-α (TNF-α)

Abcam's TNF alpha rat *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Rat TNF alpha in serum.

ASSAY PROCEDURE

Equilibrate all materials and prepared reagents to room temperature prior to use.

Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.

Determine the number of microplate strips required to test the desired number of samples, plus appropriate number of wells needed for controls and standards. Remove sufficient microplate strips from the pouch.

Add 100 ul. of each standard and sample, including blank controls to the appropriate wells.

Add 50 ul. of 1X Biotinylated anti-TNF alpha to all wells.

Cover and incubate for 3 hours at room temperature (18-25°C).

Remove the cover and wash the plate as follows:

Aspirate the liquid from each well.

Add 300 ul. of 1X Wash Buffer into each well.

Aspirate the liquid from each well.

Repeat for a total of 3 washes.

Add 100 ul. of 1X Streptavidin-HRP solution into all wells, including the blank wells. Re-cover and incubate at room temperature for 30 minutes.

Add 100 Lil. of Chromogen TMB substrate solution into each well and incubate in the dark for 1020 minutes at room temperature.

Avoid direct exposure to light by wrapping the plate in aluminum foil.

Add 100 LL of Stop Reagent into each well. Results must be taken immediately after the addition of Stop Reagent, or within one hour, if the microplate is stored at 2-8°C in the dark.

Read absorbance of each well on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm as the reference wavelength.

3.5.2 Estimation of serum superoxide dismutase (SOD) activity

Principle: Superoxide dismutase (SOD) catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen, also produces a water-soluble formazan dye upon reduction with superoxide anion. The rate of the reduction with a superoxide anion is linearly related to the xanthine oxidase (XD) activity, and is inhibited by SOD. Therefore, the inhibition of activity of SOD can be determined by a colorimetric method.

Materials and reagent

i. Microcentrifuge

ii. Pipettes and pipette tips

iii. Colorimetric microplate reader

iv. 96 well plate

v. SOD standard

Reagent composition

i. Vost solution 1ml

ii. SOD enzyme solution 20(L

iii. SOD assay buffer 20(L

iv. SOD dilution buffer 10ml

Procedure: 1ml of tissue was homogenized in ice cold 0.1m tris, pH 7.4. The crude tissue homogenate was centrifuged at 14000xg for 5minutes at 40C and the cell debris discarded and serum used.

Table 6: Amount of solution in each well

Plates were incubated at 370C for 20mintes and absorbance read at 450nm using a microplate reader.

Calculation:

$$\text{SOD Activity} = \frac{(\text{Ablank 1} - \text{Ablank 3}) - (\text{Ablank 1} - \text{Ablank 2})}{(\text{Ablank 1} - \text{Ablank 3})} \times 100 \quad (\text{Inhibition rate } \%)$$

3.6 STATISTICAL ANALYSIS

Statistical analysis was done using general descriptive statistics, One Way Analysis of Variance (ANOVA) at the $p<0.05$ significant level. If significant differences are found, LSD test was used to establish the source of significance difference. The computer software, Statistical Package for Social Scientists (SPSS) and Microsoft Excel was also used for statistical analysis.

CHAPTER FOUR

4.0 RESULTS

The result of the experiment is presented below

4.1 Tumour Necrosis Factor alpha (TNF α)

The lowest TNF α value, 87pg/ml was recorded in Group 2 on day 21. The highest value, 136pg/ml was obtained from Group 1 on day 7 of the experiment.

4.1.1 Days

The minimum TNF α value of 116.7pg/ml recorded for day 7 of the experiment was obtained from Group 4. The maximum TNF α value of 136pg/ml also recorded for day 7 of the experiment was obtained from Group 1. The mean value of TNF α calculated for day 7 was 122.1 ± 9.28 pg/ml. The lowest TNF α value of 110.5pg/ml recorded for day 14 in Group 2. The highest TNF α value of 133.6pg/ml also recorded for day 14 was obtained from Group 4. The mean value of TNF α calculated for day 14 was 123.78 ± 9.78 pg/ml. The minimum TNF α value of 87pg/ml recorded for day 21 of the experiment was found in Group 2. The maximum TNF α value of 110.5pg/ml recorded for day twenty-one of the experiment was found in Group 1. The mean value of TNF α calculated for day 21 was 96.83 ± 12.20pg/ml. The p value when comparing the Tumour necrosis factor alpha for the various days was 0.082.

4.1.2 Groups

The p value when comparing the Tumour necrosis factor alpha for the various groups was 0.445.

In Group 1, the TNF α values ranged from 110.5 to 136pg/ml. The maximum value was obtained on day 7 while the minimum value was obtained on day 21. The mean value of TNF

α calculated for Group 1 was 124.7±12.99 pg/ml. In Group 2, the TNF α values ranged from 87 to 117.6pg/ml. The highest value was obtained on day 7 while the minimum value was obtained on day 21. The mean value of TNF α calculated for Group 2 was 105.03 ± 16.01 pg/ml. The mean value of TNF α in the blood serum was calculated for Group 3 as 111.52 ± 16.25 pg/ml. The minimum value recorded was 93pg/ml on day 21 while the maximum value recorded was 123.45pg/ml on day 14. The minimum TNF α value recorded in Group 4 was 116.7 pg/ml on day 7 while the maximum value recorded was 133.6 pg/ml on day 14. The mean value recorded was 125.15 ± 11.95pg/ml (Fig 4.2).

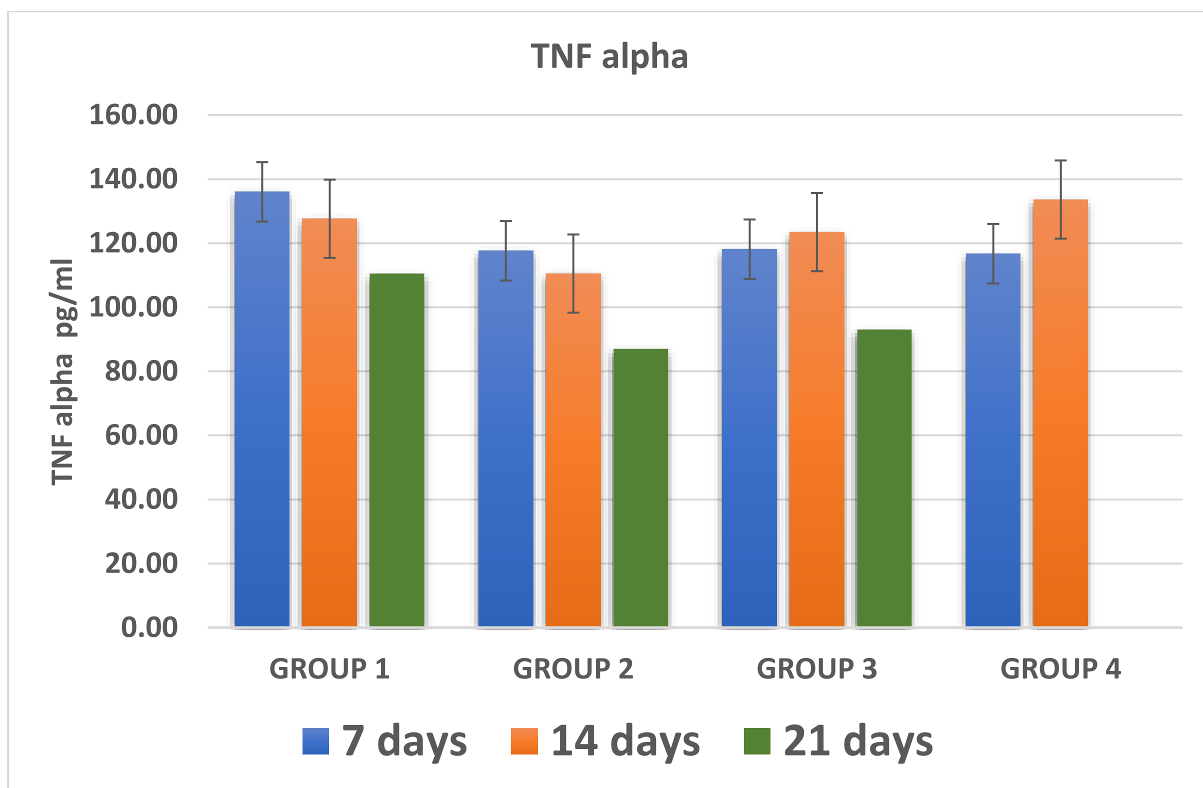

Figure 4.1 Tumour Necrosis Factor alpha values of experimental animals.

4.2 SUPEROXIDE DISMUTASE (SOD)

Throughout the experiment, Group 2's SOD value peaked at 25.15 U/mg of protein on Day 21 of exposure, while Group 1's SOD value peaked at 12.6 U/mg of protein on Day 7 of exposure. The superoxide dismustase was compared using p values of 0.887 and 0.334, respectively, for different days and groups.

4.2.1 Days

The rats that were not given any treatment had the lowest SOD value (12.6U/mg protein) for day 7 recorded. The group of rats given cicatrin powder treatment had the highest SOD value (17.85 U/mg protein) on day 7. The computed mean value of MDA was 14.86 2.19 U/mg protein. For day 14, the SOD levels ranged from 14.75 to 18.45 U/mg protein. The lowest SOD value was found in Group 1, while the highest value was found in Group 2. The computed mean value of SOD was 16.59 1.56 U/mg protein. The computed day 21 mean value of SOD was 22.67 3.28 U/mg of protein. Group 1 provided the lowest SOD value (18.95 U/mg protein observed for day 21). The Group 2 group had the highest SOD value on day twenty-one, which was 25.15 U/mg protein.

4.2.2 Groups

The lowest observed SOD value in Group 1 was 12.6 U/mg protein, while the highest value was 18.95 U/mg protein. These numbers were noted on days seven and twenty-one, respectively. SOD was measured on average at 15.43 3.22 U/mg protein. Group 2 measured SOD values as low as 17.85 U/mg of protein and as high as 25.15 U/mg of protein, respectively. These values were acquired on days 7 and 21 of the experiment, respectively. The reported median value was 20.48 4.05 U/mg protein. For Group 3, the mean value was 18.38 4.98 U/mg protein. With the lowest and highest values obtained on days 7 and 21, respectively, the SOD levels varied from 14.2 to 23.9 U/mg protein. SOD levels in Group 4

ranged from a minimum of 14.8 U/mg protein on day 7 to a maximum of 16.1 U/mg protein on day 14. The average result was 15.45 0.92 U/mg of protein (Fig.4.4).

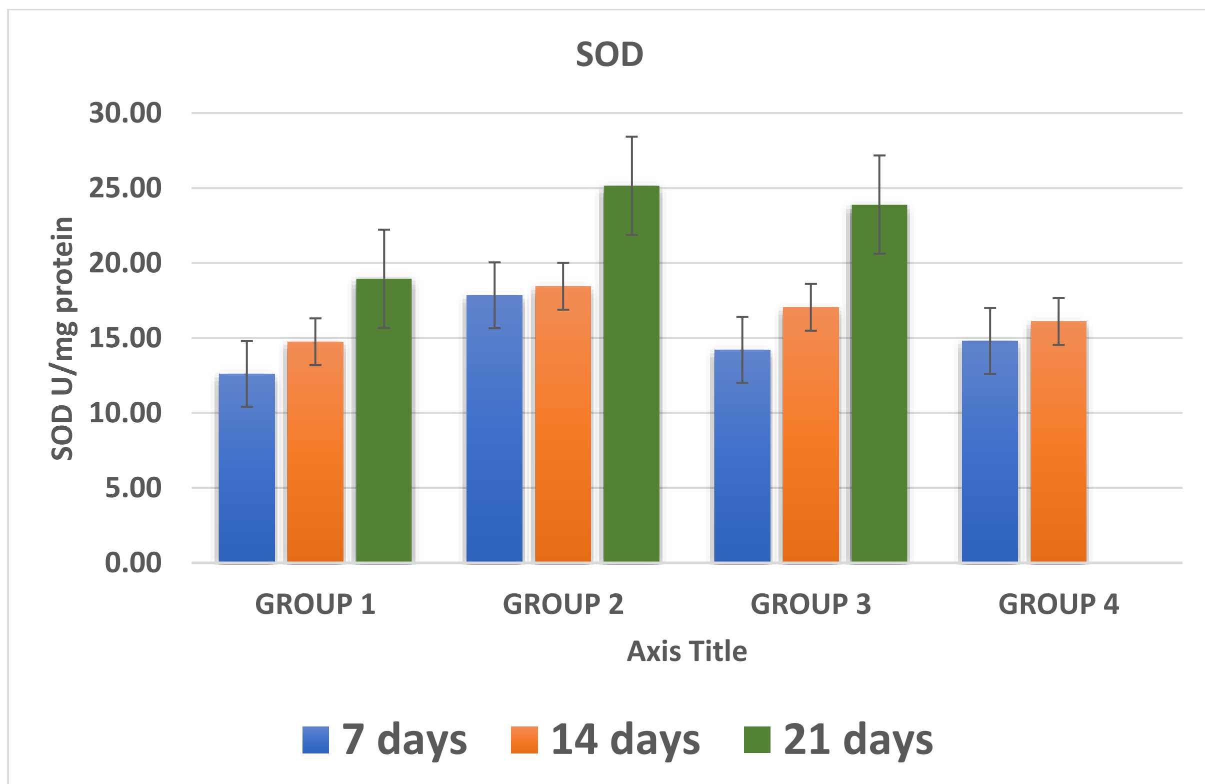

Figure 4.2 **Superoxide dismutase (SOD) values of experimental animals.**

CHAPTER FIVE

5.0 DISCUSSION

Enormous reports demonstrate that plants from genus *Parkia* possess medicinal values, attributable to the presence of pharmacological active compounds. Taken together, two most studied species, *P. biglobosa* and *P. speciosa*, show potential as antidiabetic, antihypertensive, and antimicrobial, to name a few (Ntui *et al.,* 2012). The results of this study shows that the effect of ethanol extracts of *P. biglobosa* on wounded healing animals revealed that tumour necrosis factor (TNF-α) of group 1 animals range from 136.0 pg/ml to 110.5 pg/ml, group 2 animals range from 87.0 pg/ml to 117.6 pg/ml, group 3 animals range from 123.45 pg/ml to 117.6 pg/ml, while group 4 animals range from 116.7 pg/ml to 133.6 pg/ml. However the maximum value was obtained on day 7 while the minimum value was obtained on day 21. This was in line with the study of Costa *et al.* (2013) who stated that TNF values for group 1 and 2 animals treated with *Parkia* species ranged from 124.5 – 138.9. According to Ogunyinka *et al.* (2016) tumor necrosis factor is an adipokine and a cytokine. TNF is a member of the TNF superfamily, which consists of various transmembrane proteins, promotes insulin resistance, used by the immune system for cell signalling. If macrophages (certain white blood cells) detect an infection, they release TNF to alert other immune system cells as part of an inflammatory response (Mondal *et al.,* 2013). Ogunyinka *et al.* (2017) further confirmed that TNF signalling occurs through two receptors: TNFR1 and TNFR2. TNFR1 is constitutively expressed on most cell types, whereas TNFR2 is restricted primarily to endothelial, epithelial, and subsets of immune cells (Sheikh *et al.,* 2016). TNFR1 signalling tends to be pro-inflammatory and apoptotic, whereas TNFR2 signalling is anti-inflammatory and promotes cell proliferation. Suppression of TNFR1 signalling has been important for treatment of autoimmune disease, whereas TNFR2 signalling promotes wound

healing (Builders *et al.,* 2021). This could be the mechanism behind the wound healing ability of experimental animals induced by *P. biglobosa* ethanol extract.

The results of this study also shows that the Superoxide dismutase (SOD) values of the treated animals were as follows: group 1 animals range from 12.6 U/mg to 18.95 U/mg, group 2 animals range from 17.85 U/mg to 25.15 U/mg, group 3 animals range from 14.2 U/mg to 23.9 U/mg, while group 4 animals range from 14.8 U/mg to 16.1 U/mg. However the maximum value was obtained on day 21 while the minimum value was obtained on day 7. This also in line with the work of Boye *et al.* (2016) who reported that SOD values for group 2 and 3 animals treated with *P. speciosa* ranged from 14.8 – 18.6, while group 4 and 5 treated animals ranged from 15.3 – 17.1. According to Builders (2012) SOD is an enzyme that alternately catalyzes the dismutation of superoxide radical, produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage. Thus, it is an important antioxidant defense in nearly all living cells exposed to oxygen, thereby inhibiting endothelial activation (Ong *et al.,* 2012), as it was seen in the present study. The study of Soetan *et al.* (2011) showed that SOD out-competes damaging reactions of superoxide, thus protecting the cell from superoxide toxicity. The reaction of superoxide with non-radicals is spin-forbidden. In biological systems, this means that its main reactions are with itself (dismutation) or with another biological radical such as nitric oxide (NO) or with a transition-series metal (Iyamu *et al.,* 2014).

However, in mice inactivation of SOD2 causes perinatal lethality and inactivation of SOD1 causes hepatocellular carcinoma according to Boye *et al.* (2016). Furthermore SáSantos *et al.* (2018) explained that the physiological importance of SODs is illustrated by the severe pathologies evident in mice genetically engineered to lack these enzymes. Mice lacking SOD2 die several days after birth, amid massive oxidative stress (Mondal *et al.,* 2013). Mice lacking SOD1 develop a wide range of pathologies, including hepatocellular carcinoma, an

acceleration of age-related muscle mass loss, an earlier incidence of cataracts, and a reduced lifespan. Mice lacking SOD3 do not show any obvious defects and exhibit a normal lifespan, though they are more sensitive to hyperoxic injury (Fayinminnu *et al.,* 2017).

Conclusion

The wound-healing activity of *P. biglobosa* ethanol extract in a simple experimental model has been established. Further studies are recommended to isolate and characterize the relevant bioactive components and elucidate the mechanisms of actions of these active ingredients. The potential and safety of this plant extract, as well as their possible protective mechanisms, should be determined before administration into humans. However, further research on the bioactive compounds present in the plant extract should not be excluded, as the discoveries of novel agents from this plant could provide an alternative to the treatment of surgical wounds.

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Appendix I – Result of Biomarkers

Results for Tumour Necrosis Factor alpha (TNF-α)

Groups	7 days	14 days	21 days
1	136	127.6	110.5
2	117.6	110.5	87
3	118.1	123.45	93
4	116.7	133.6	-

Results for Superoxide Dismutase (SOD)

Groups	7 days	14 days	21 days
1	12.6	14.75	18.95
2	17.85	18.45	25.15
3	14.2	17.05	23.9
4	14.8	16.1	-

Appendix II- Data Analysis

ANOVA: TWO-FACTOR WITHOUT REPLICATION FOR TUMOUR NECROSIS FACTOR ALPHA

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
GROUP 1	3	374.1	124.7	168.87
GROUP 2	3	315.1	105.0333	256.5033
GROUP 3	3	334.55	111.5167	264.3058
GROUP 4	3	250.3	83.43333	5292.243
7 days	4	488.4	122.1	86.20667
14 days	4	495.15	123.7875	95.83062
21 days	4	290.5	72.625	2443.563

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	2670.587	3	890.1958	1.025923	0.445149	4.757063
Columns	6757.633	2	3378.816	3.893983	0.082405	5.143253
Error	5206.212	6	867.702			
Total	14634.43	11				

ANOVA: TWO- FACTOR WITHOUT REPLICATION FOR SUPEROXIDE DISMUTASE

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
GROUP 1	3	46.3	15.43333	10.43083
GROUP 2	3	61.45	20.48333	16.42333
GROUP 3	3	55.15	18.38333	24.85583
GROUP 4	3	30.9	10.3	79.99
7 days	4	59.45	14.8625	4.828958
14 days	4	66.35	16.5875	2.432292
21 days	4	68	17	135.6117

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	175.505	3	58.50167	1.386768	0.334567	4.757063
Columns	10.28625	2	5.143125	0.121917	0.88736	5.143253
Error	253.1138	6	42.18563			
Total	438.905	11				

Appendix III – Ethical Approval