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# *In vivo* study on the effect of African black tea extract on wound healing

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**Abstract**
**Background:** The use of alternative medicine is on the rise worldwide. Black tea (*Camellia Sinensis*) is believed to assist in wound healing among communities who apply it topically. This had however not been scientifically proven.
**Objective:** To investigate the *in vivo* effects of black tealeaves extracts on different stages of wound healing from haemostasis to remodelling.
**Methodology:** Following acclimatization, twenty-eight male pure breed Albino Wistar rats were randomly divided into two major groups, an excisional group (n=22) and an incisional group (n=6). The animals were anaesthetized, their dorsums shaved to expose the surgical site and antisepsis performed. Two 4 cm long, full thickness, incisional wounds (control and experimental) were inflicted on the respective groups. The incisions were then closed using nylon 2-0 interrupted sutures to allow healing by primary intention. Two circular full thickness excisional wounds (control and experimental) each measuring 4 mm in diameter were inflicted on the excisional wounds group, and allowed to heal by secondary intention. Postoperatively, analgesia and prophylactic antibiotic was provided to prevent infection. They received daily 100 mg/ml aqueous extract of black tea on experimental wounds and distilled water on control wounds. Bleeding time, clotting time, coagulation profile, biometric measurements of wound closure, histomorphological analysis, and tensile strength of the incision wounds were measured.
**Results:** Black tea extract significantly lowered the clotting time and activated partial thromboplastin time in comparison to the control. The experimental wounds containing the extract demonstrated faster rates of closure, re-epithelialization, matrix deposition and remodelling. The experimental wounds also had a higher mean tensile strength on days 15 and 30 compared to the control. The extract did not significantly affect the bleeding time and mean prothrombin time of the experimental wounds.
**Conclusion:** African black tea enhances wound healing and could potentially be a good source of wound healing compounds.

**Keywords:** *Camellia sinensis*, wound healing, haemostasis, re-epithelialization, remodelling

## Introduction

The skin is considered the largest organ in the human body composed of two main layers, the epidermis and dermis [1]. It bears crucial functions including protection, thermoregulation, fluid and electrolyte regulation, somatosensation, and metabolism. Its damage leads to the loss of such function [2]. Such damage, commonly referred to as wounds, can broadly be classified into acute and chronic [3]. Wounds are a major concern in medicine with the rapidly rising incidence rates. The commonest etiological types include surgical wounds, estimated at over 100million annually worldwide followed by traumatic wounds estimated at over 50million, chronic wounds at over 40million, and burn wounds making up over 10million. Wounds follow a standard healing sequence of physiological processes that comprise coagulation, inflammation, fibroplasia, matrix deposition, contraction, angiogenesis, epithelialization, and remodelling. This healing sequence can take place via primary intention, delayed primary intention or secondary intention depending on wound edge proximity [4].

The use of alternative medicine is on the rise worldwide despite the availability of conventional pharmaceutical products for various reasons including cultural beliefs, easy availability, lack of access to modern medication, and low cost [5]. Their therapeutic claims and efficacy are however seldom investigated. It is estimated that at least 80% of Africans prefer traditional medicine for the treatment of common ailments [6]. It would therefore be expedient to complement, rather than to supersede such customs.

One such traditional practice is the use of tea (*Camellia Sinensis*) in the treatment of wounds. Tea is the second most popular drink worldwide. *Camellia sinensis* was first used to produce tea more than 57 centuries ago in China, and has since become popular all over the world [7].

The chemical components of tealeaves include inter-alia alkaloids (including caffeine, theophylline, and theobromide), polyphenols (catechins and flavonoides), and inorganic elements (including fluoride and aluminium). The polyphenols, catechins and flavonoids are however the main health beneficial components. The flavonoids have many therapeutic properties including anti-inflammatory, antioxidant, anti-microbial and anti-allergic effects. Catechins compounds include catechin, epicatechin, gallocatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate, with the latter being the most active [8].

White and green tea have been shown to enhance wound healing [9, 10]. However, little effort has been made to determine the effect of black tea, which has a different composition from white and green tea, on wound healing and elaborate on its mechanism of action. Anecdotal beliefs recommend the use of black tea to enhance wound healing, by application of either dry tea leaves or in aqueous solution on the site of injury [11]. This belief; however, has no scientific evidence. This study therefore aimed to investigate the *in vivo* effect of Black tea *(Camellia sinensis)* leaves extract on wound healing patterns and investigate its mechanism of action.

## 2. Materials and methods

### 2.1 Animals

Twenty eight 3-4 month old male pure breed Albino Winstar rats weighing 120-140 g were acquired from University of Nairobi's department of Medical Physiology animal unit. They were fed *ad libitum* on commercial pellets, provided free water supply, kept at standard laboratory conditions, and housed in separate cages. Exclusion criteria included disease. One-week acclimatization period was allowed for the animals before experimentation. The animals were handled with care and in accordance with the internationally accepted principles for laboratory animal use and care of Federation of European Laboratory Animal Science Association (FELASA) guidelines. Ethical approval was obtained from the Bioethics, Animal Use and Ethics Committee, Faculty of Veterinary Medicine, University of Nairobi.

### 2.2 Wound models

The rats were maintained in good health and just before wounding, they were examined for any clinical signs of disease for exclusion. General anaesthesia was induced using inhalational halothane (dose dependent on the animal's response). The rats' dorsum was shaved using a hair calliper to expose the surgical sites. Antisepsis of the area was performed using 70% ethanol.

Incisional wounds were inflicted on the incisional wounds group of animals (n = 6). Two full thickness incisions, one experimental and the other control, measuring 4 cm long each, were made 2 cm paravertebral from the midline as described by Ehrlich and Hunt [12]. The incisions were then closed using nylon 2-0 vertical reverse mattress interrupted sutures, 1 cm apart, to provide the tensile strength to allow mobilization without wound breakdown.

Excisional wounds were inflicted on the relevant group of animals (n=22). Two standardized full thickness excisional wounds, one experimental and the other control, each measuring 4 mm in diameter, were inflicted 2 cm paravertebral on each of the animals using a punch biopsy, dissecting forceps and scissors by a modified method described by Morton and Malone [13].

Following surgery, the animals were monitored for full recovery from anaesthesia on a warm blanket. Analgesia was provided using intramuscular 10mg/kg diclofenac and prophylactic 0.1 ml of 100000IU penicillin-G antibiotic provided to avoid infection [14]. The animals were, thereafter, housed in separate cages to avoid them from interfering with one another's wounds.

### 2.3 Extract preparation & intervention

Processed black tealeaves was purchased from commercial suppliers (Ketepa Tea Packers) and extraction was done by dissolving a known amount in a known volume of distilled water, to achieve a concentration of 100mg/ml. The animals received daily wound care during which 100 mg/ml aqueous solutions of *Camellia sinensis* was applied on the experimental wounds (The dose was determined using preliminary tests) and distilled water of equal volume applied to the control wounds.

### 2.4 Biometric analysis of wound closure

The progression of wound repair and closure was monitored daily for 30 days post operatively. The wounds were measured using a digital caliper by measuring the largest ($D_1$) and smallest ($D_2$) diameters. Wound area was subsequently calculated as the area of an ellipse.

Where; Area of an eclipse = $\boldsymbol{\pi} \times (D_1 \div 2) \times (D_2 \div 2)$

Wound closure was thereafter calculated as percentage reduction in wound area as described by Hajiaghaalipour *et al.* [9]

$$\text{Contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

### 2.5 Bleeding time

This test measures the time taken for vasoconstriction and platelet plug formation to occur. No clot is allowed to form, so that the arrest of bleeding depends exclusively on blood vessel constriction and platelet action [15]. Following the excisional wounding as described above, a gauze soaked in 2ml of 100mg/ml black tea extract and another soaked in distilled water were used to directly damp the experimental and control wounds bleeding sites respectively as described by Kochan *et al*. [16] The time it took for each wound to stop bleeding was recorded as the bleeding time.

### 2.6 Clotting time

The time taken for blood to clot reflects the time required for the generation of thrombin. If the plasma concentration of prothrombin or of some of the other factors is low (or if the factor is absent, or functionally inactive), clotting time will be prolonged. Whereas, if the factors are enhanced, the time will be shortened [17].

Following the incision of wounds as described above, blood was obtained to measure clotting time using a modified capillary tube method described by Mayer [18].

For the control group, a plain capillary tube was filled with blood, and immediately thereafter the tube was broken at regular intervals and the time taken for a fibrin thread to appear was noted down. For the experimental group, capillary tubes that had earlier been soaked in 100mg/ml of black tea extract, drained, and allowed to dry were used to perform the above procedure.

### 2.7 Coagulation profile

Coagulation profile was tested using an automated ACL 7000 © coagulation analyser to test for Activated Partial Thromboplastin Time (aPTT) and Prothrombin Time (PT). The PT measures the integrity of the extrinsic pathways (factors VII, V, X, prothrombin, and fibrinogen); whereas, aPTT measures the intrinsic pathway [17].

Animals that had reached the end of their experimentation and were to be euthanized for tissue harvesting were bled for coagulation profile first. Anaesthesia was induced using inhalational halothane, and 4 ml blood was collected from each animal by intracardiac phlebotomy into citrate blood vacutainers. Automated coagulation profiling was run after adding 0.1 ml of 100mg/ml black tea extract for experimental sample. This was then compared against the profile of the control samples where the extract is not added and the same volume of distilled water used instead.

### 2.8 Tensile Strength of Incision Wound Model
Following euthanasia of the animal, the maximum load (tensile strength) tolerated by the incision wounds was measured by applying a measurable load on opposite direction of the incision wounds following removal of the nylon sutures until disruption occurs. The load was measured in Newtons (N). This was done on days 15 and 30.

### 2.9 Histomorphometric analysis of wound healing
Following euthanasia of 3 excisional animals per day on days 3, 5, 7, 15 and 30, tissues from the healing excision wounds were harvested by wide excision around them and fixed immediately in 10% neutral-buffered formalin. The tissues were thereafter processed, embedded, blocked and cut into 7μm sections using a Leitz Wetzar ® rotary microtome, perpendicular to the wound surface.

The sections were thereafter mounted and stained with Masson-trichrome stain for examination and photography, using a Leica® DM 500 light microscope, of re-epithelialization, collagen formation, fibroblast proliferation, angiogenesis, granulation tissue formation, and remodelling.

### 2.10 Data and statistical analysis
The recordings generated above were analysed for percentage difference between the control and experimental groups in the various parameters. The data was expressed as mean ± SEM. Suitable inferential statistic tests were employed to test significance with the P value set at <5%. Statistical Package for Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, Illinois) was used to analyse the data.

## 3. Results
### 3.1 Bleeding time
The bleeding time of the experimental wounds had a mean of 11.8 ± 1.36 seconds; whereas, that of the control had a mean of 11.4 ± 1.29 seconds. The difference was not significant as shown by Mann-Whitney U test (U calc = 12.5; P (u) = 1.00). These results are summarized in figure 1.

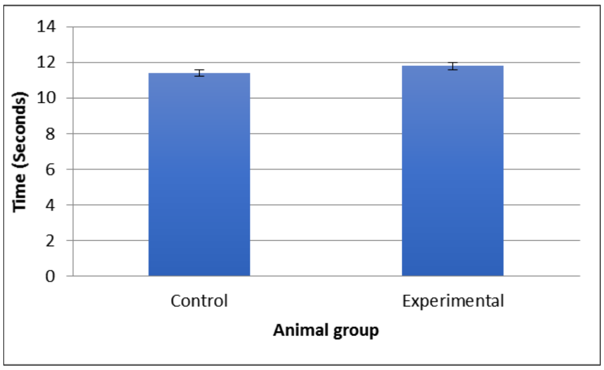



**Fig 1:** Bar graph showing the effect of black tea on bleeding time. P = 1.00

### 3.2 Clotting time
The clotting time of the experimental wounds had a mean of 48.8 ± 4.62 seconds; whereas, that of the control wounds had a mean of 79.2 ± 6.4 seconds. A test to compare the two revealed that the difference was highly significant as shown by Mann-Whitney test (U calc = 0.00; P (u) = 0.009). This is as presented in figure 2.

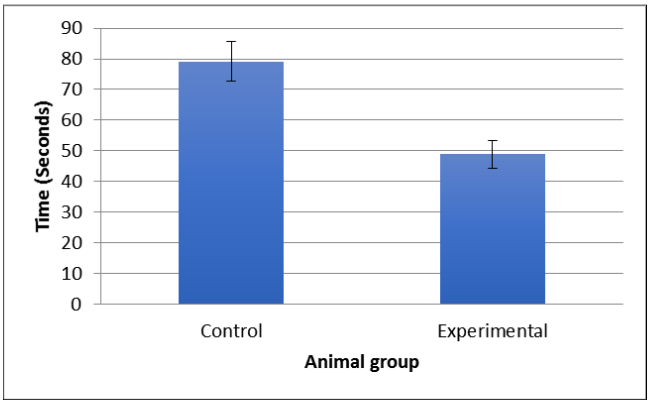



**Fig 2:** Bar graph showing effect of black tea on clotting time. P = 0.009

### 3.3 Activated Partial Thromboplastin Time
The Activated Partial Thromboplastin Time (aPTT) of the experimental blood samples treated with black tea extract had a mean on 9 ± 0.95 seconds; whereas, that of the control samples had a mean of 22.5 ± 0.29 seconds. A test to compare the two revealed that the difference was significant as shown by Mann-Whitney test (U calc = 0.00; P (u) = 0.024). This is illustrated in figure 3.

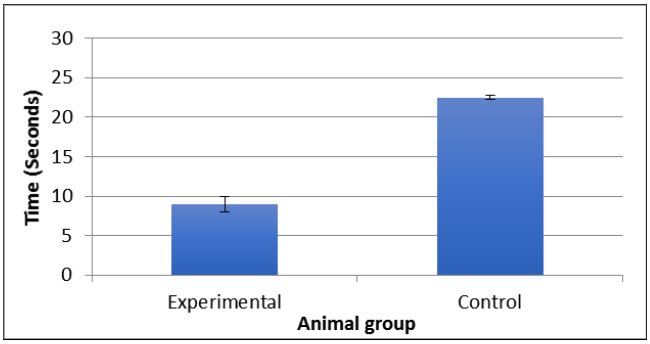



**Fig 3:** Bar graph showing the effect of black tea on aPTT. P = 0.024

### 3.4 Prothrombin Time
The Prothrombin Time (PT) of the experimental blood samples, treated with black tea extract had a mean on 8.6 ± 0.75 seconds; whereas, that of the control samples had a mean of 8.2 ± 0.52 seconds. A test to compare the two revealed that the difference was not significant as shown by Mann-Whitney test (U calc = 6.00; P (u) = 0.653). This is summarized in figure 4.

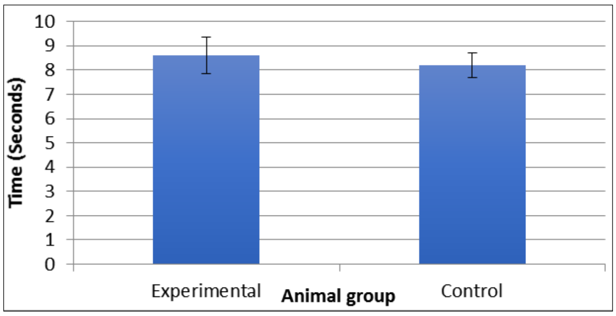



**Fig 4:** Bar graph showing effect of black tea on PT. P = 0.653

### 3.5 Effect on wound closure
Figure 5 displays the progressive wound closure of both experimental and control wounds. There was an initial

increase in wound area, and therefore a negative percentage closure for both wounds, followed by progressive positive percentage closure. The experimental wounds were consistently ahead of the control wounds till complete closure on day 15. The difference however tested statistically insignificant using Chi-square test ($\chi^2$ = 28.00; P ($\chi^2$) = 0.411).

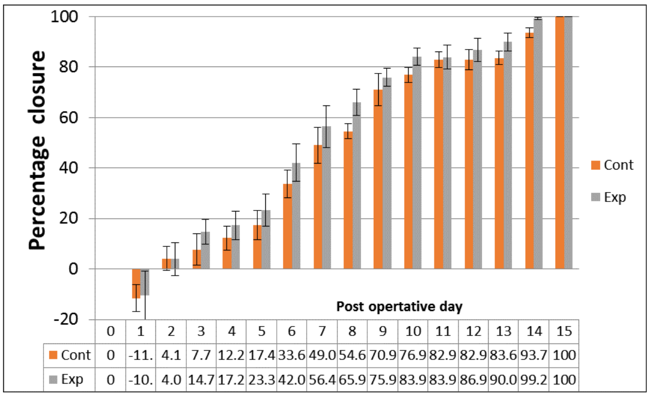
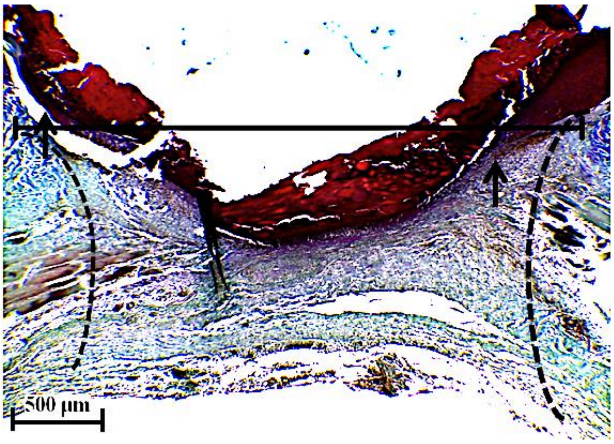
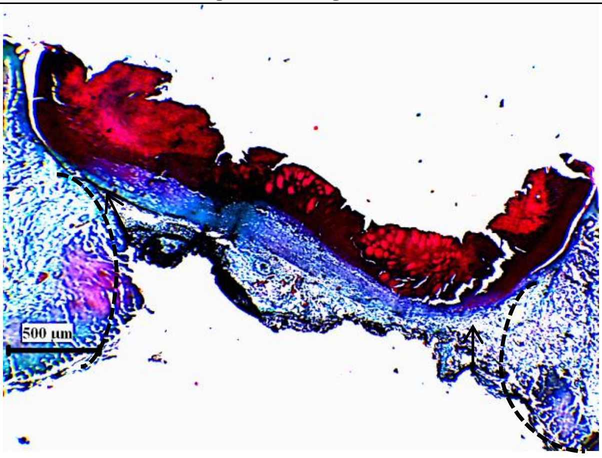
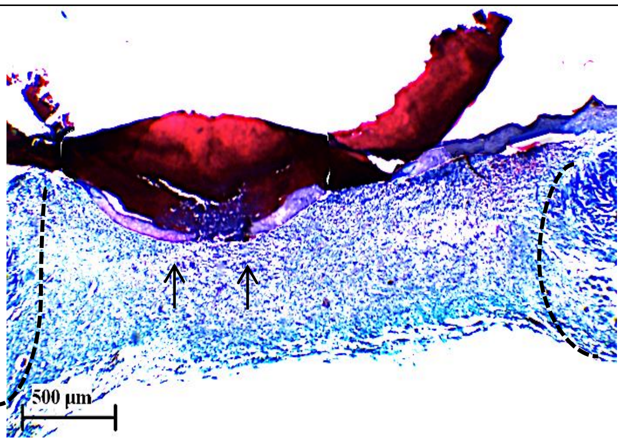
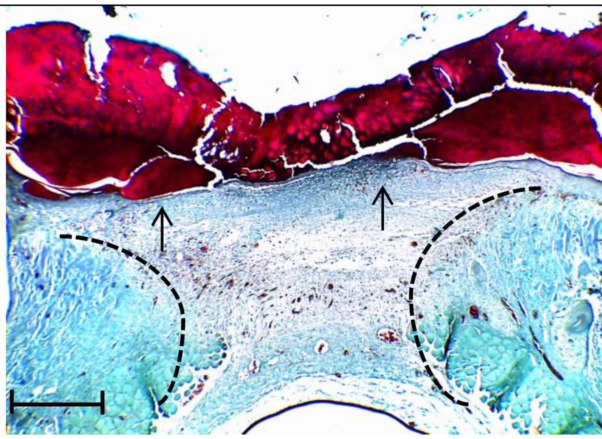



**Fig 5:** Bar graph showing the effect of black tea on wound closure on control and experimental wounds. P = 0.411

### 3.6 Histomorphometric assessment of the effect of black tea of wound healing

Figure 6 shows the histomorphometric findings of the effect of black tea on wound healing.

| Day | Experimental | Control |
|---|---|---|
| P.S Arrow showing the tip of neo-epithelium while dotted line showing wound edges. | | |
| 3 |   Average wound diameter: 3059.2 µm<br>Distance migrated by epithelium: 1097.1 µm<br>Percentage epithelialization: 35.87% |   Average wound diameter: 3193.61 µm<br>Distance migrated by epithelium: 335.97 µm<br>Percentage epithelialization: 10.52% |
| 5 |   Average wound diameter: 2368.01 µm<br>Distance migrated by epithelium: 2120.33 µm<br>Percentage epithelialization: 89.54%<br><br>Epidermis not fully re-epithelialized. Dermis appears to have more collagen deposition in the experimental wound. |  Average wound diameter: 3036.84 µm<br>Distance migrated by epithelium: 1702.46 µm<br>Percentage epithelialization: 56.06%<br><br>Epidermis not fully re-epithelialized. Dermis appears to have less collaged deposition compared to experimental wound with more porous areas. There is also evidence of a higher presence |

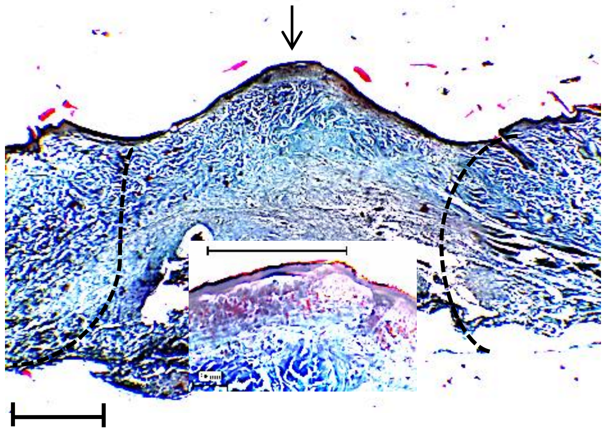
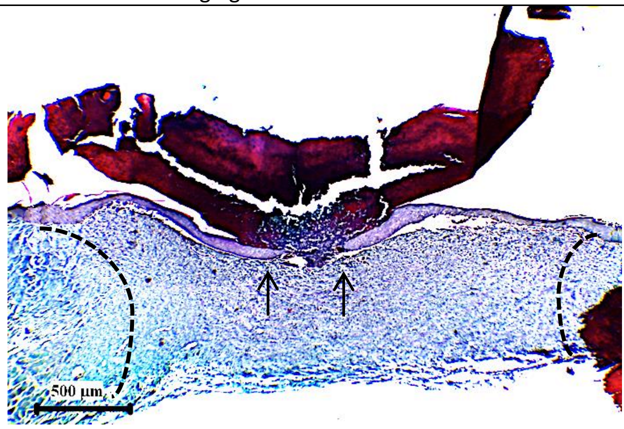
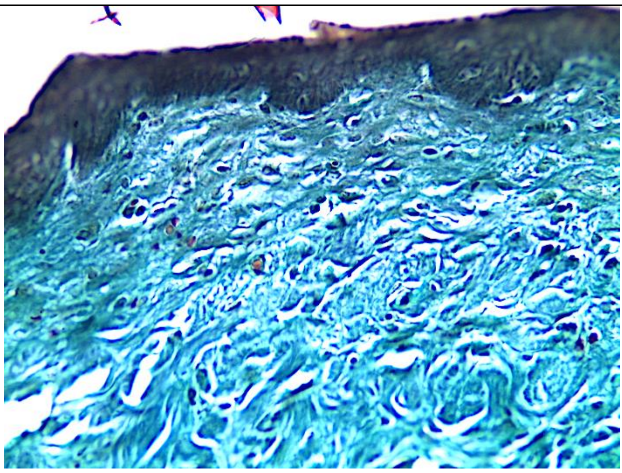
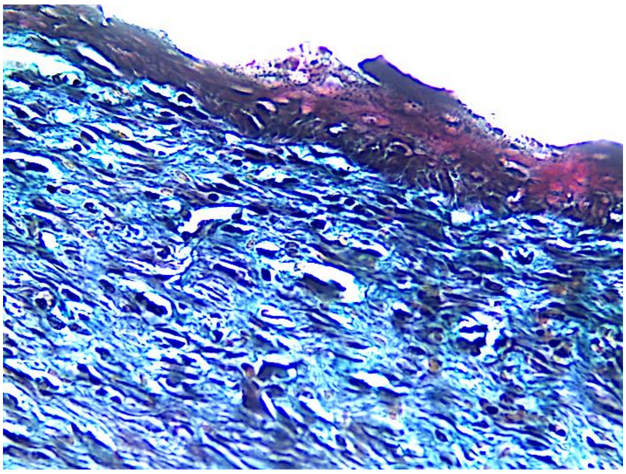
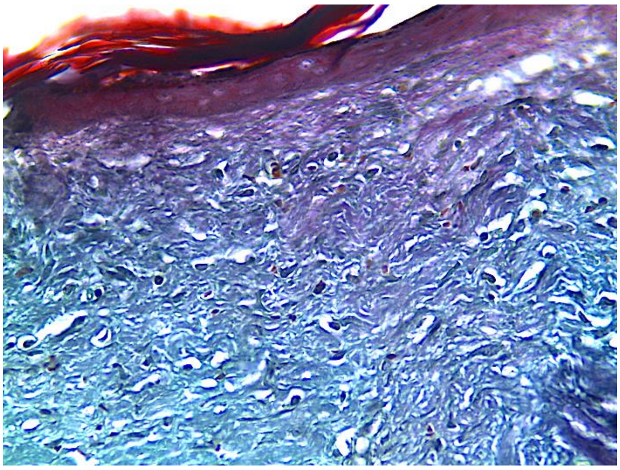
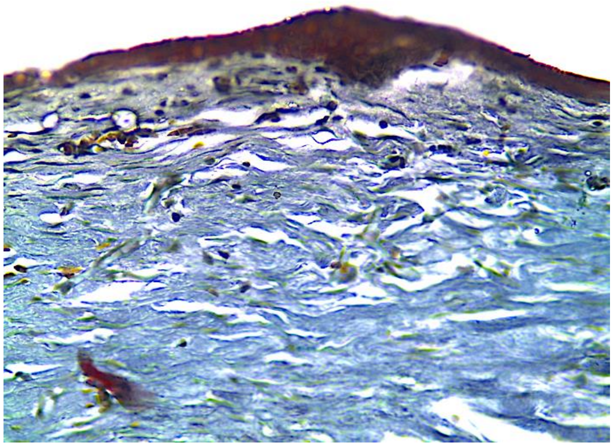
| | | |
|---|---|---|
| | | of neo-angiogenesis on the control wound. |
| 7 | <br>Average wound diameter: 2579.21 µm<br>Distance migrated by epithelium: 2579.21 µm<br>Percentage epithelialization: 100%<br><br>Epidermis fully reepithelialised, and the dermis appears to have dense collagen deposition with the fibres arranged in a basket woven orientation. | <br>Average wound diameter: 2057.69 µm<br>Distance migrated by epithelium: 1766.88 µm<br>Percentage epithelialization: 85.87%<br><br>Epidermis not fully reepithelialised, and the dermis has collagen laid in spicules bearing granular orientation |
| 15 | <br>Dense collagen bearing basket woven orientation, with lower cell population | <br>collagen fibres more linear on orientation, with a higher cell population |
| 30 | <br>Collagen more densely packed in a basket woven orientation | <br>Collagen less densely packed in linear orientation. Higher presence of porous areas |

**Fig 6:** Histomorphometric assessment of effects of black tea on wound healing

### 3.7 Effect on wound strength

On day 15, the tensile strength of the experimental incisional wounds, treated with black tea extract, had a mean of 7.35± 1.94 Newtons; whereas, that of the control wounds had a mean of 4.02 ± 1.20 Newtons. Furthermore, on day 30, the tensile strength of the experimental incisional wounds, had a mean of 20.59 ± 3.29 Newtons; whereas, that of the control wounds had a mean of 16.67 ± 2.68 Newtons. This is shown in figure 7.

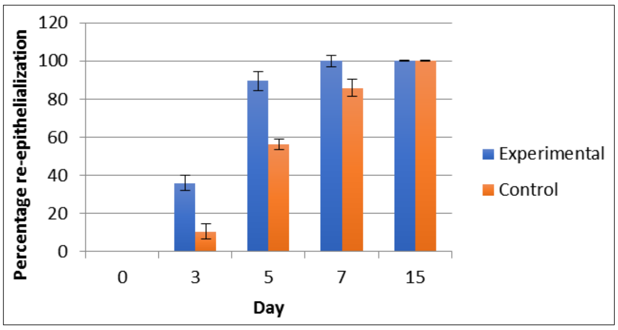



**Fig 7:** Bar graph showing effect of black tea on wound strength

## 4. Discussion

The use of alternative medicine is on the rise worldwide [19]. Many cultures have beliefs and prejudices, towards the treatment of various morbidities that often affect their attitudes towards conventional medical practices. At least 80% of Africans prefer traditional medicine for the treatment of common ailments [6]. It would therefore be expedient to supplement, rather than to supplant such beliefs and cultural customs. This is the basis of ethnopharmacology, a relatively new science, which has over the years been focussing on evaluating indigenously used drugs [20].

Medicinal plants are considered as an important source of potentially useful structures for the development of new chemotherapeutic agents, and the main aim is to validate (or invalidate) the plants through the isolation of their active substances [20]. Many plants have been proven effective and are being recommended for use throughout the world. Furthermore, the easy availability, low cost, and belief of fewer associated side effects are the major attractions to herbal medicine among such communities [6].

One such traditional practice is the use of various plant constituents to accelerate wound healing as well as to improve its quality. Wound healing progression is comprised of stages that commence from the moment of injury, that is, the inflammatory phase (haemostasis and inflammation), the proliferation phase (granulation, contraction, and epithelialisation), and finally, the remodelling phase, which determines the strength and appearance of the healed tissue.

Other than being the second most popular drink worldwide [7], tea *(Camellia sinensis)* is also a major commercial crop that is available widely across the globe. Its ready availability makes it a suitable herbal/traditional treatment modality to various ailments [6].

The phytochemical composition of tea leaves include polyphenols (catechins and flavonoids), alkaloids (caffeine, theobromine, theophylline, etc.), volatile oils, polysaccharides, amino acids, lipids, vitamins (e.g., vitamin C), and inorganic elements (e.g., aluminium, fluorine, and manganese). Tea leaves have also been shown to contain various types of tannin [21]. The polyphenols are the groups of compounds that are primarily responsible for the therapeutic properties of tea [8]. Flavonoids are a group of plant metabolites that are believed to provide health benefits through cell signalling pathways and antioxidant effects. They have the general structure of a 15-carbon skeleton, which consists of two benzene rings linked together by a heterocyclic ring. They possess, inter-alia, antioxidant, anti-inflammatory, anti-allergic, and antimicrobial effects [22]. Catechins, which belong to a group of flavonoids known as flavanols, are made of two benzene rings bearing a dihydropyran heterocycle with a hydroxyl group attached to carbon 3 [23].

Tea is believed to enhance wound healing, a conviction that is commonly promoted among communities [11]. Studies have been conducted on other varieties of tea, including green and white tea, suggesting efficacy. It has been shown that oral consumption of green tea enhances the initial stage of re-epithelialization, although the enhancement is not as evident on later stages of healing [10]. Furthermore, topical application of green tea has been shown to reduce healing time [24]. Topical application of white tea has also been demonstrated to enhance healing in that it enhances wound closure [9].

Studies on *Camelia sinensis* have, however, been limited to wound closure, and no studies have targeted the whole spectrum of wound healing which runs from haemostasis to remodelling. Furthermore, no studies have been conducted on black tea, which has been shown to have a different phytochemical content.

Haemostasis is the initial step of wound healing, which can be life saving in severe cases of trauma. It is the mechanism that prevents the loss of blood, a vital body fluid. It is the first physiological response that occurs following trauma and begins with vasoconstriction and platelet plug aggregation and, thereafter, clotting factors coagulation. This study has demonstrated that black tea has no significant effect on bleeding time and, therefore, probably, neither affects vasoconstriction nor platelet plug formation. It, however, significantly lowered clotting time, which means that it probably enhances blood coagulation. Coagulation profile was conducted using an automated machine in order to minimize human error that may have occurred during coagulation time testing using capillary tube method. This showed that black tea significantly reduced Activated Partial Thromboplastin time (aPTT) but has no significant effect on Prothrombin Time (PT) demonstrating that its effect on coagulation is via the intrinsic pathway. Tea has been reported to contain tannins which accelerate blood clotting [25]. It is, therefore, via tannins that black tea, probably, is a pro-coagulant.

The current study has also demonstrated that black tea enhances the proliferative phase of wound healing, which consists of granulation, epithelialisation, and contraction (26). On macroscopic assessment, there was an initial increase in the excisional wound area, probably due to necrosis of tissue that was damaged during the wounding process. Thereafter the experimental wounds showed some increase in the rate of closure compared to the control wounds throughout the experimentation period. The increase, however, tested statistically insignificant. Studies have shown that the Panniculus carnosus, a striated muscle found in the hypodermis of rodents, and absent in humans, rapidly contracts, following wounding [27]. This probably overshadows the proliferative wound closure under investigation, hence testing insignificant.

Furthermore, on microscopy, the experimental wounds consistently showed faster re-epithelialization with a complete epithelium noted on day 7 post-wounding. The control wounds extended beyond 7 days to fully epithelialize. The epithelium was easy to note as it clearly stains purple with Masson trichrome stain. Angiogenesis was also noted to have a higher presence on the experimental wound, where a higher microcapillary presence was noted. Angiogenesis is important in wound healing, as it is the new vessels that transport wound healing cells to the wound site so as to participate in the healing process. It was, however, not possible to objectively quantify the vessels, as that would require specialized immunohistochemistry, staining to tag for endothelial cells, and electron microscopy.

The intensity of collagen staining (green) was consistently higher in the experimental wounds. Collagen staining intensity is a measure of synthesis density as described by Hajiaghaalipour *et al.* [9]. The fibre orientation was also noted to be basket woven on the experimental wounds. This indicated a more advanced stage of wound healing, a stage that occurs during remodelling where linear collagen III fibres are replaced with mechanically robust basket woven collagen I fibres. In addition to dense, basket woven collagen deposition, was a lower cellularity (presence of fibroblasts) as compared to the control wounds. Fibroblasts reduce in population, through apoptosis, as they progress to deposit extra-cellular matrix, composed mainly of collagen, at a healing site. These features suggest accelerated healing on the experimental wounds. To further support enhanced experimental wound strength, considered a measure of advanced remodelling, the experimental wounds tested significantly stronger when exposed to tensiometry.

Studies have demonstrated that Transforming growth factor-beta1 (TGF- β1), a polypeptide cytokine, is expressed during the various stages of wound healing. TGF- β1 stimulates fibroblast proliferation and differentiation and extracellular matrix synthesis, as well as wound contraction [8]. Alpha 3 beta 1 (α3 β1) integrin, a laminin receptor, also enhances re-epithelialization as it acts as a receptor for basement membrane [29]. Epigallocatechin-3-gallate (EGCG), which is contained in tea, has been shown to enhance the role and expression of TGF- β 1 and α3 β1 [30].

Leu *et al*. also demonstrated that EGCG enhances the expression of vascular endothelial cell growth factor and angiopoietin-1 protein expression, both of which are protein messengers that enhance angiogenesis in wound healing [31]. This leads to increased neo-angiogenesis as demonstrated by the current study.

Reactive oxygen species (ROS) are harmful to body cells and are also detrimental to wound healing [32]. Non-polymeric phenolic and polymeric tannin are contained in *camellia sinensis* and have been demonstrated to have antioxidant properties [33]. Furthermore, EGCG has been shown to possess anti-oxidant properties [31]. Anti-oxidation; therefore, plays a crucial role in the process of wound healing by protecting the regenerated tissue from the harmful oxidative damage of ROS, a mechanism that probably contributes to the enhanced wound healing by black tea.

Non-polymeric phenolic and polymeric tannin also bear antibacterial properties against Gram positive bacteria (33). Tea, especially when applied topically on to wounds; therefore, has the potential of preventing infections that commonly lead to wound chronicity.

The enhanced wound healing action of black tea may; therefore, be via multiple mechanisms. Its phytochemical constituents may be agonistic to important mediators of haemostasis and wound proliferation; they may also have free radical scavenging activity which is protective to regenerated tissues; they may also be antibacterial which prevents infections, a common cause of wound chronicity.

The bioavailability of catechins following oral administration of tea is 50 times less than that of topical administration [34]. Furthermore, topical administration of TGF- β1 or its agonist is more effective in enhancing healing [35]. Topical administration of black tea is, therefore, the most effective way to harness its wound healing properties.

## 5. Conclusion

The current study is in keeping with various scientific as well as anecdotal beliefs, and proposes that African Black tea enhances wound healing and could potentially be a good source of wound healing compounds.

## 6. Conflict of interest declaration

We declare that we have no conflict of interest. This manuscript/data or parts thereof, has not been submitted for possible publication to another journal or previously been published elsewhere.

## 7. Acknowledgment

The authors acknowledge the department of Medical Physiology, University of Nairobi, where this study was conducted.

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