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Wound Healing and Curative Properties of Alligator Pepper (*Aframomum Melegueta*) seed extract in Albino Rats (*Rattus Norvegicus*)

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ABSTRACT: The aim of this study was to determine the effects of Alligator pepper, *Aframomum melegueta*, seed extract on wound healing in albino rats. Four treatment groups of eight albino rats each were used for the experiment. Group 1 were given feed and water only. Group 2, 3 and 4 were exposed to a reference drug (Cicatrin), 10% and 20% of the extract respectively for 21 days. Excision wounds of 30mm were made on dorsal thoracic central region of the rats. Serum from blood collected from the dorsal aorta of sacrificed rats were analysed for oxidative stress biomarkers (TNF-α, MDA and SOD) using standard methods. The tissue samples from the wounds were collected and histhopathologically analysed under a light microscope. The decrease in wound size was measured using an electronic Vernier Calliper at intervals of two days. The values of TNF-α ranged from 87pg/ml to 136pg/ml while those of MDA ranged from 0.92 to 3.19µmol/L. The maximum and minimum values of SOD recorded were 25.15U/mg protein and 12.6U/mg protein respectively. The smallest wound size value, 5.5mm, was measured in Group 3 on day 19 of the experiment. Behavioural changes were observed which included lethargy and anorexia. The histopathological study of the extract groups showed the formation of new tissues, keratinized substances and hair follicle. The results obtained from the wound measurements supported the conclusion that the extract had a better effect on the wound healing in experimental animals.

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Keywords: Alligator pepper, wound, healing, albino rat, cicatrin,

A wound is a disorder of the cellular, functional and anatomical continuity of a living tissue. Kumarasamyraja *et al*., 2012 best describes it as the foremost case of physical disabilities. With the infliction of wound on an individual, healing follows. Wound healing basically involves the process of repairing the injury to the skin and other soft tissues. *Aframomum melegueta* is a tropical herbaceous perennial plant. It is a plant which is often eaten as a snack among the elderly in Nigeria and its seeds possess a sharp peppery taste, a property that is due to its aromatic ketones. The plant is a very popular spice used mainly as food, in brewing, and in veterinary and traditional medicine. Phytochemical analysis of *Aframomum melegueta* showed that the plant is rich in phenol and tannins as well as other constituents that supports and reinforces its use in numerous works in the medical field (Alaje *et al*., 2014). These constituents indicate a couple of things including the fact that the plant might be a good antimicrobial agent, act as an anti-inflammatory, anticlotting and immune enhancer and also prevent the formation of wound infections and also the treatment of these wounds at a faster pace. Tannins were also found in high amount in the phytochemical analysis of *Aframomum melegueta.* It has a stringent properties, quickens the healing of wounds and inflamed mucous membrane. Hence, it further supports the use of the wound healing potential of the plant (Alaje *et al.*, 2014). The aim of this study was to determine the effects of Alligator pepper seed, *Aframomum melegueta*, extract on wound healing in albino rats and for the extract to serve as a natural alternative for wound healing.

MATERIALS AND METHODS

Plant materials: The seeds of *Aframomum melegueta* were purchased from Watts Market in October 2019 and identified by Dr. Ekpo of the Department of Botany, University of Calabar, Calabar, Nigeria.

Preparation of extract: Seeds of *Aframomum melegueta* were dried and pulverized. 800g of the pulverized dried seeds was extracted exhaustively by cold maceration in 80% methanol for 48 hours. This was filtered with a Whatmann No. 1 filter paper. The filtrate was concentrated with a rotary evaporator at 40 ^{o}C. The extract was stored in a refrigerator at 4 ^{o}C until when used. Two types of ointment formulations were

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prepared from the extract: 10% and 20% (w/w), where 10 or 20g of the extract was incorporated into 100g of simple ointment, sterile white paraffin base respectively.

Animals: The animals were divided into four groups with eight animals in each and kept in plastic cages. Animals had access to standard pellet feed and water *ad libitum*. The study protocol was approved by the Institutional Ethical Review Committee, Life Sciences, University of Benin, Benin City, Edo State.

Wound Healing Studies: Experimental animals obtained from the breeding unit of the Department of Anatomy, Faculty of Basic and Medical Sciences, University of Benin, Benin City. They were kept in plastic cages with wire mesh covers on the cages and were fed with rat pellet and water *ad libitum* for a one week acclimatization period. Wheat straws were used as bedding materials for the cages. The animals were divided into four groups of eight animals each. The first two group served as control while the other groups were treated with the extract.

Excision Wound Model: Excision of wounds were made as described by Morton and Malone, 1972. Animals were placed on the operation table in natural position and were anaesthetized intraperitoneal with a mixture of ketamine and xylazine. Hairs were removed from the dorsal thoracic central region of anaesthetized rats. A circular wound of about approximately 30mm was made on depilated ethanol-sterilized dorsal thoracic region of rats.

Treatment Protocol: While one treatment group was given food and water only for twenty-one (21) days, the other three groups, in addition to being fed with food and water, had their wounds treated with either the reference drug or the extract. All preparations were applied topically, once daily for 21 days in the following manner: Group 1 (Control) – normal excision wounded animal given food and water only, Group 2 (Standard drug) – normal excision wounded animal treated with cicatrin powder, Group 3 (Test 1) – Normal excision wounded animal treated with the 10% *Aframomum melegueta* seed extract, Group 4 (Test 2) – Normal excision wounded animal treated with the 20% *Aframomum melegueta* seed extract. At the end of a 7 days period, samples of needed tissues and blood were collected and subsequently analysed for histopathological changes and haematology respectively and also the overall effects of the alligator pepper extract on wound healing in albino rats. Wound Contraction (which is a major contributor to wound closure) was studied during the course of the experiment. The wound healing area of each animal was measured post wounding days for its contraction with the aid of an electronic calliper. An interval of two days was given between each wound contraction measurement. Blood serum samples were collected for haematological analysis. Tissues samples of the healing wounds were also obtained for histological analysis.

Determination of oxidative stress biomarkers: Determination of oxidative stress biomarkers (TNF-α, MDA and SOD) were carried out according to the methods previously described by Airaodion *et al*. 2019 and Khalifa, *et al*., 2020).

Statistical Analysis: Data collected from the study were analysed using general descriptive statistics, two Way Analysis of Variance (ANOVA). Values of the measured parameters were expressed as mean value ± SD and the statistical analysis were done with the aid of Statistical Program for Social Sciences (SPSS) and Microsoft Excel.

RESULTS AND DISCUSSION

The results of the experiment clarified that the *Aframomum melegueta seed* extract had effects on the wound of albino rats. This was measured in terms of behavioural changes, changes wound area, histopathology and biomarkers used to measure oxidative stress. *Behavioural changes*: Experimental animals from Group 4, which were exposed to the 20% *Aframomum melegueta* seed extract showed signs of anorexia and lethargy during the period of observations. These signs followed with death of four individuals from this exposure group. It is suggests that further experiment be performed with larger number of rats to eliminate the possibility of losing all individuals in an exposure group before the experiment is terminated.

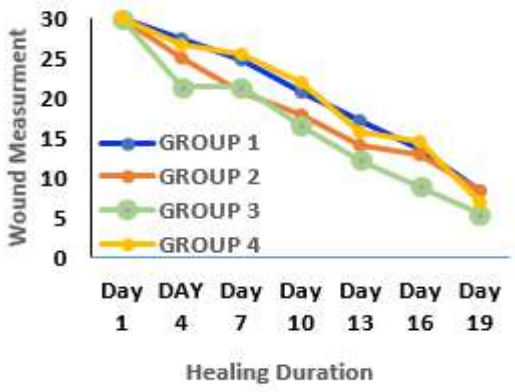
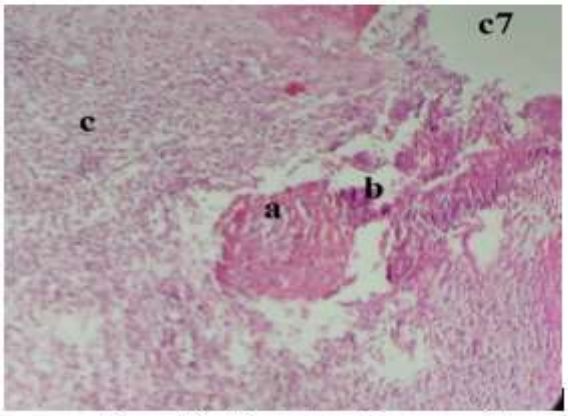


Fig. 1: Wound measurement values of experimental animals

Wound Healing Observation: The results of the wound area measurements in this study showed that there was

significant difference between the different treatment groups ($p < 0.05$). In this study, there was a drastic reduction in the healing time and an enhanced rate of decrease in wound area, when the rats were exposed to 10% *Aframomum melegueta* seed extract when compared with that of the control (Fig. 1). There was also a significant difference in the healing time and rate of decrease in wound size when the 10% extract group was compared to the experimental animals exposed to the reference drug, Cicatrin.

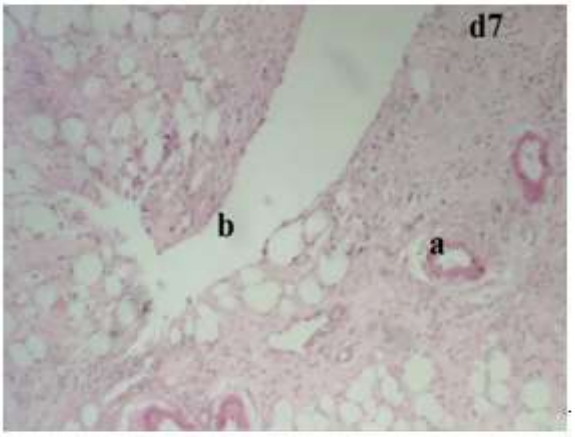
Rathi *et al.* (2006) stated the significant importance of wound contraction in healing of excised wounds. And El-Ferjani *et al*. (2016) concurred with this stating that it contributes to restoration of the cellular structure in damaged tissues. Results obtained in the present study suggested that treatment of excision wound with extracts of *Aframomum melegueta* accelerated the wound healing process as compared to the control treatment. The wound healing potential of the extract may be attributed to the presence of the mixture of phytoconstituents including flavonoids, tannins and phenols. Nagar *et al*., 2015 suggested that the better wound contraction found in a group of the experimental animal models was attributed to the presence of phenolic compounds which was also found in both *A. melegueta* seed extract.



a - some tissue defect, b - extra skin, c - exudates

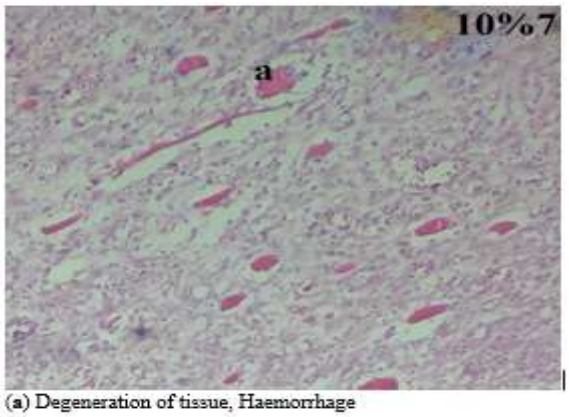
Plate 1: Photomicrograph of wound in the control group on day 7

Histopathological studies were carried out on the excised wounds from all the treatment groups. The study revealed that the wounds treated with 10% extract showed the degeneration of tissue, formation of new epidermis and hair follicle as the wound healing progressed (Plate 3, 7 and 11). Collagen fibres and keratinized substances were seen as well from viewing with the light microscope. The studies of the tissue samples from 20% extract group showed keratinization and collagen fibres formation (Plate 8). Animals treated with the cicatrin drug showed the presence of angiogenesis, collagen fibre formation and a dilated lumen (Plate 2, 4, 6 & 10). The control group showed normal histopathology all through the experiment (Plate 1, 5, 9*)*.



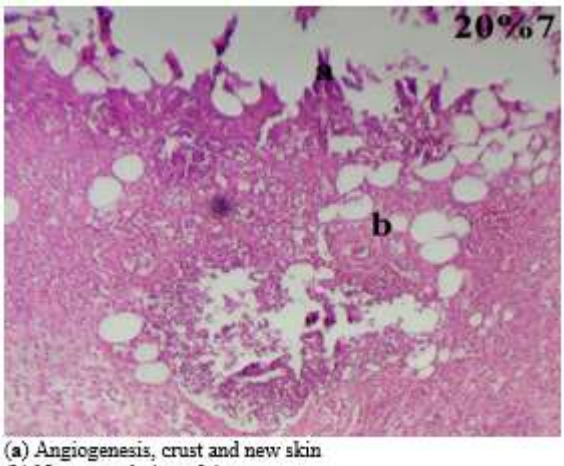
(a) There is presence of angiogenesis, new epidermis
(b) New granulation of tissue with vesicles

Plate 2: Photomicrograph of wound treated with standard drug on day 7



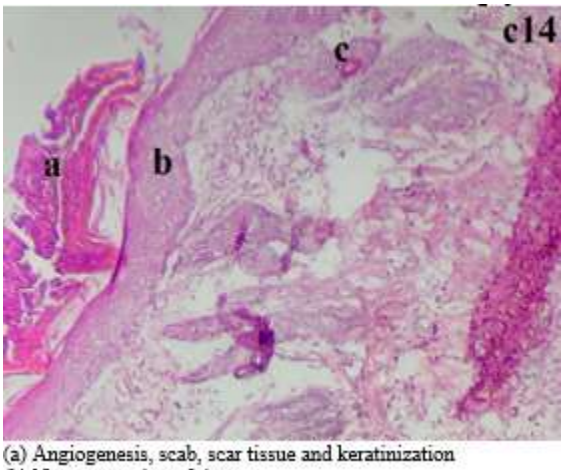
(a) Degeneration of tissue, Haemorrhage

Plate 3: Photomicrograph of wound treated with 10% extract on day 7



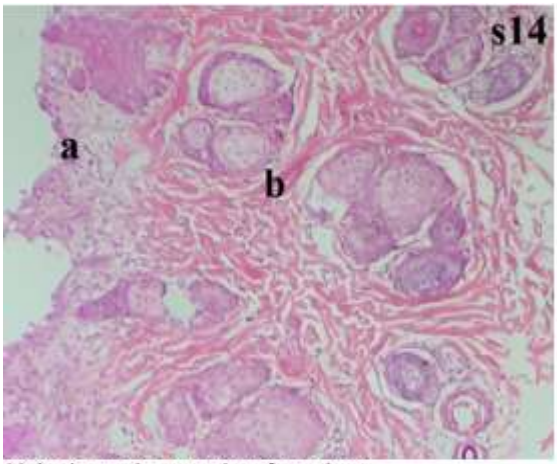
(a) Angiogenesis, crust and new skin
(b) New granulation of tissue

Plate 4: Photomicrograph of wound treated with 20% extract on day 7



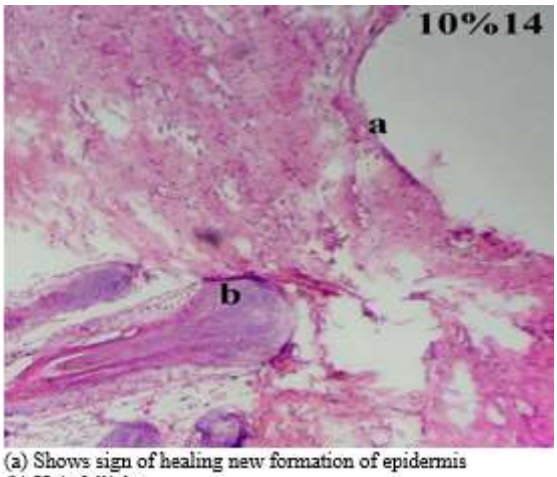
(a) Angiogenesis, scab, scar tissue and keratinization
(b) New generation of tissue

Plate 5: Photomicrograph of wound in the control group on day 14



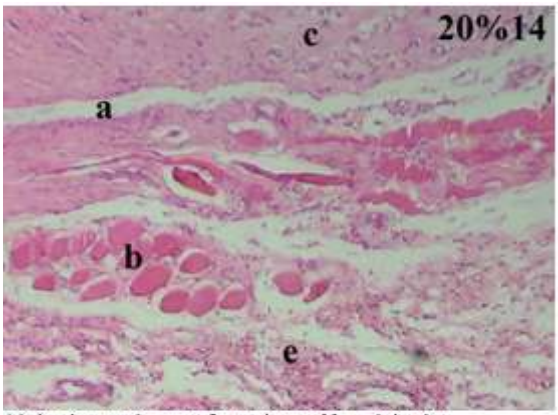
(a) Angiogenesis, generation of new tissues
(b) Collagen fibres formation

Plate 6: Photomicrograph of wound in the Standard drug group on day 14



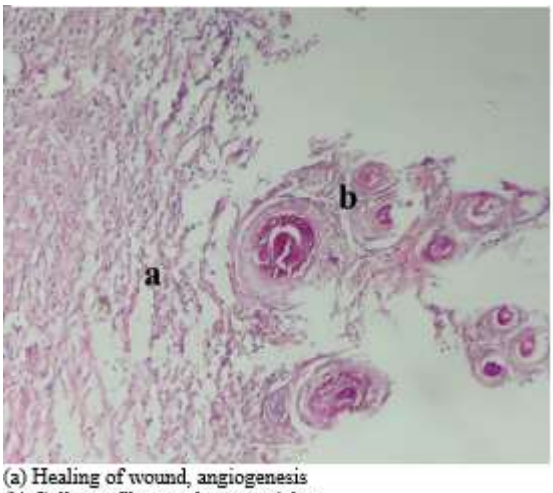
(a) Shows sign of healing new formation of epidermis
(b) Hair follicles

Plate 7: Photomicrograph of wound in the 10% extract group on day 14



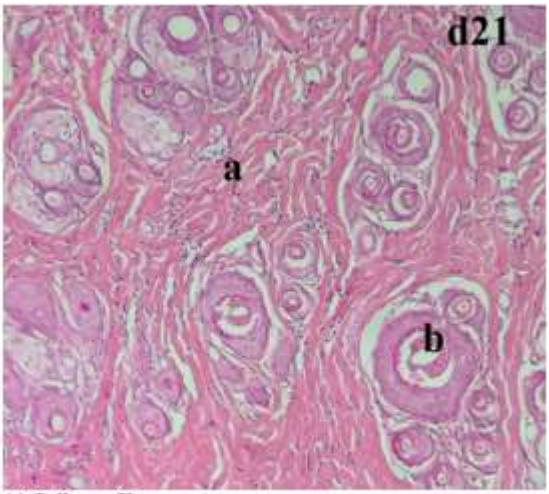
(a) Angiogenesis, crust formation and keratinization
(b) Collagen fibres and formation of new

Plate 8: Photomicrograph of wound in the 20% extract group on day 14



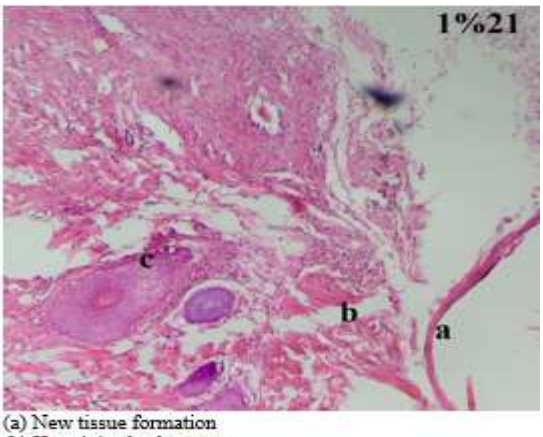
(a) Healing of wound, angiogenesis
(b) Collagen fibres and new vesicles

Plate 9: Photomicrograph of wound in the control group on day 21



(a) Collagen fibres
(b) Capillaries and dilated lumen with fibrin

Plate 10: Photomicrograph of wound in the standard drug group on day 21



(a) New tissue formation
(b) Keratinized substances
(c) Hair follicles

Plate 11: Photomicrograph of wound in the 10% extract group on day 21

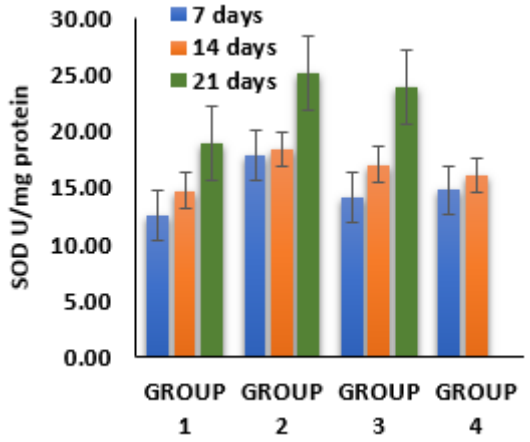


Fig. 2: Superoxide dismutase (SOD) values of experimental animals

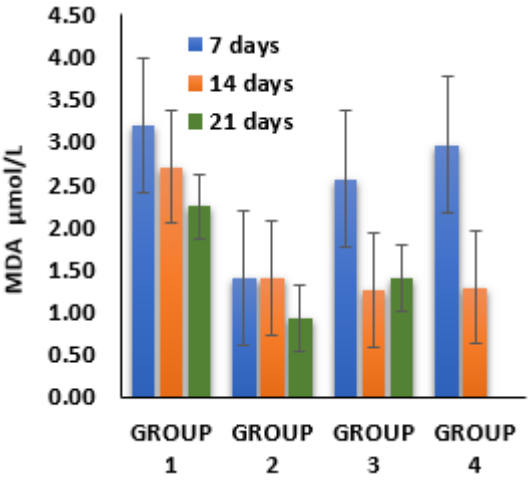


Fig. 3: Malondialdehyde (MDA) values of experimental animals

Injury to the skin induces intricate reactions, including early inflammation during which free radicals and oxidants are excessively released in to the wound area. These free radicals which are released during the process of inflammation can cause severe oxidative damage to the healing wound. Endogenous oxidants like SOD in granulation tissues accelerate the process of wound healing by neutralizing the free radicals. Therefore, cutaneous wounds must be properly protected against excessive inflammatory cell influx and free radical-mediated process in cutaneous wound. Estimation of the biochemical antioxidant, Superoxide dismutase, was carried out in this study to determine the effect of the extracts on the oxidative stress levels and also to be able to compare its values in the various experimental groups (Fig. 2). The results showed that there was a higher production of SOD in the treatment group given the extract as compared to the control group. This was also in correlation with the group that had the highest rate of wound decrease (10% *A. melegueta seed* extract). It also suggests that the extract possessed antioxidant property which would help to prevent oxidative damage and promote wound healing process. Several studies indicate that SOD appears to be useful in improving the rate of wound healing. These include the work of Kurahashi and Fujii (2015), Schäfer and Wemer (2008). The result of this study also corresponded with Agarwal *et al.* (2009) study which involved the evaluation wound healing activity of extracts of plant banana (*Musa sapientum* var. *paradisiaca*) in rats. The MDA levels in all groups of this study showed that the *A. melegueta* seed extract had less oxidative stress when compared with the serum MDA in the control group (Fig. 3). This was seen in the case of the 10% and 20% extract. This indicates that the extract lead to the reduction in lipid peroxidation that releases MDA known to cause worsening of tissue injury in high amounts.

Reduction in Malondialdehyde (MDA) levels in experimental animals when exposed to extract of plants and other chemicals has been reported by several authors. Serarslan *et al*. (2007) suggested that Caffecic acid phenetyl ester had effects in the acceleration of wound healing because of the significant decrease in MDA levels amongst others. This indicated that a decrease in MDA levels showed that a particular injury was healing. This deduction was also in accord with the work of Lovric *et al*. (2008). Another biomarker that can be used to assess the state of a wound is the pro inflammatory marker, Tumour necrosis factor-α. During the wound healing process, the peak of TNF-α production was found in the group experimental animals left untreated (control group) (Fig. 4). This was greater than those of the animals in all the treatment groups on day seven. When compared with the results of wound size, the control group showed the slowest growth as well. This suggests that a high value TNF-α indicated a higher oxidative stress level which delays the rate of wound

healing. Various reports such as that given by Goel *et al*. (2010), described that TNF-α is known to stimulate the process of angiogenesis which is the formation of new blood vessels and the build-up of tissue granulation bed. This invariably suggests that it has significant potential to improve the healing process. Hence, experimental animals that have been exposed to extract and subsequently shows an increase in the rate of TNF-α production will evidently produce better wound healing results than those left to heal with any treatment application to their wounds.

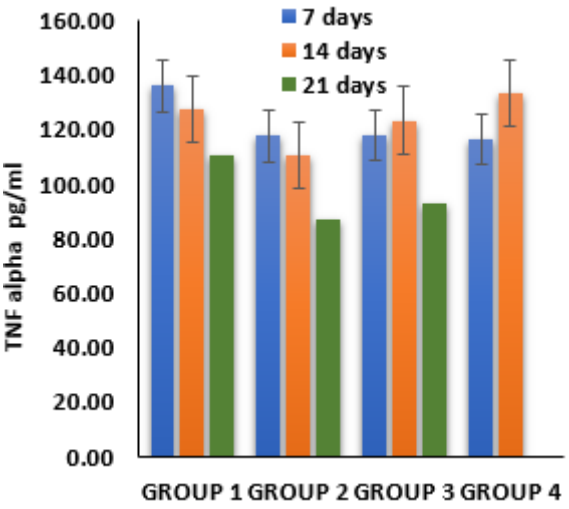


Fig. 4: Tumour Necrosis Factor alpha values of experimental animals

This contradicted the results from the present study with the *A. melegueta seed* extract as a faster reduction in the wound size was found in the extract groups which showed lower TNF-α values. Ritsu *et al*. (2017) study on the critical role of tumour necrosis factor-α in the early process of wound healing in the skin. The result obtained in their research showed that TNF-α assisted in the acceleration of the wound repair on the third day of the experiment but did not on day seven of the fourteen day experiment. A conclusion suggesting that the marker plays a crucial role in the early process of wound healing was made at the end of the study.
Mittal *et al.* (2009) work suggested that faster wound contraction would be seen in experimental groups where there is inhibition of the pro inflammatory marker, TNF-α which known to affect the function and recruitment of various inflammatory cells, fibroblasts and keratinocytes. The present study agrees with this work done.

Conclusion: The results produced from this study points to the fact that the plant, alligator pepper, indeed contains wound healing properties. Further studies on this should therefore be encouraged as it will prove useful in the area of public health and the world at large.

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