Wound Healing Activities of Latex of Grassleaf Spurge 
*(Euphobia graminea Jacq.)*

Morakinyo A.E.1,*1, Akinpelu B. A.2, Bolaji A. O.3, Adelowo J. M.4, Babarinde S. O.1, 
Orimolade O. O.3, Oladimeji O. D.3, Adewola O. O.3, Oyedapo O. O.3

1Department of Biochemistry, Adeleke University, Ede, Osun State  
2Department of Biochemistry and Molecular Biology, Obafemi Awolowo University, Ile-Ife, Osun State  
3Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State  
4Department of Basic Sciences, Adeleke University, Ede, Osun State  
*Corresponding author: adetounadejumobi05@gmail.com

Received July 30, 2020; Revised September 01, 2020; Accepted September 10, 2020

Abstract The present study evaluated the wound healing potentials of *E. graminea* (EG) latex. The latex of EG was screened for the presence of phytochemical constituents; *in vitro* antioxidant property and erythrocyte membrane stabilizing activity was used to investigate anti-inflammatory property of the latex. The wound healing potential of *E. graminea* latex (EGL) was investigated via incision and dead space wound models in wistar rats; eighteen wistar rats (separately for each model) were randomly divided into 3 groups (6 in each group): Group A: wound + EGL; Group B: wound + Gentamycin (G); Group C: wound without treatment (control). Wound breaking strength (Incision model), hexuronic acid and hexosamine concentrations, activities of antioxidants biomolecules (superoxide dismutase, catalase, and reduced glutathione) as well as the levels of free radicals (nitric oxide-NO, malondialdehyde-MDA) were evaluated in the granulation tissue (dead space model) of experimental rats. The result of the study revealed that EGL tested positive for the presence of saponins, alkaloids, triterpenes, flavonoids and cardiac glycosides. Also, EGL contained an appreciable concentration of total flavonoids and phenolics. The latex exhibited mild ferric reducing power, inhibited DPPH in dose dependent manner as well as protected red blood cells against hypotonic and heat induced lyses. In wound incision model, EGL exhibited 13.6 % increase in wound breaking strength when compared to the control animals. Also, in dead space wound model, there was significant increase (p < 0.05) in hexosamine, hexuronic acid and GSH concentrations as well as SOD activity in EGL and gentamycin treated wound compared to the control. Furthermore, there was a significant reduction (p < 0.05) in MDA concentration in EGL and gentamycin treated wound compared to the control while NO level and catalase activity showed no significant difference. This study revealed that EGL contained potentially bioactive molecules that could be employed in the treatment of wound.

Keywords: *euphorbia graminea*, latex, wound, healing


1. Introduction

Wounds arise as a result of injuries to the skin that disrupt the soft tissue. It may be produced by the physical, chemical or microbial damage to the tissue. Wounds represent a significant burden on the affected and the healthcare professionals worldwide [1,2,3]. Wounds are the major causes of physical disabilities and affect the physical and mental health problems. Studies have revealed that nearly 6 million people suffer from chronic wounds worldwide [4]. This has drawn the attention of many scientists to identify potential means and sources of wound care.

There are a number of plants which are used traditionally for wound healing. Several plants’ extracts are known as a promising alternative for wound healing agents due to the presence of various bioactive substances, accessibility and little/no side effects [5]. Currently available therapies do not give an effective impact on tissue repairing process; hence instigate a need to develop new approach of natural origin to tissue repairing. Among such plants is *E. graminea*. It is very morphologically variable, as discussed by [6]. The possible anthropogenic uses of EGL include: treatment of skin infections such as ulcers, cancers, tumors, warts, and other diseases as practiced by Colombian traditional healers [7].

The plant belongs to the kingdom Plantae (plants), class of Magnoliopsida (dicotyledons), order of Malpighiales,
family of Euphorbiaceae (spurge family), genus of *Euphorbia* L. (spurge). It is an annual plant, generally from 15-30 cm tall, sometimes taller, often branching from the base, and dichotomously branched distally. The leaves are alternate; those toward the base of the plant are ovate or oblong with a few large distantly-spaced teeth, while those toward the top of the plants are elliptical to linear and entire [6]. Cyathia are in short- to long-pedunculate cymes and have white petaloid appendages. The capsules are exerted from the cyathia, glabrous, and about 2 x 3 mm. The seeds are angular, ovoid, ca. 1.5 x 1.3 mm, gray or whitened, and rugose [7]. There is no scientific information documented on the latex of *E. graminea*, hence this study evaluated the wound healing activity of grassleaf spurge latex in albino rats.

2. Materials and Methods

2.1. Collection of Plant Latex

*E. graminea* plant was collected from the Botanical Garden, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. It was identified at IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife. The latex was obtained from the plant into several petri-dishes, covered and kept in the freezer for biochemical analyses.

2.2. Reagents and Chemicals

All reagents and chemicals used were of analytical grades, and were purchased from Sigma Chemical Company, Germany.

2.3. Phytochemical Screening of *E. graminea* Latex

The phytochemical analysis of the latex was carried out by a procedure that was based on the earlier reports of [8,9].

2.4. Evaluation of In Vitro Antioxidant Properties of *E. graminea* Latex

2.4.1. Estimation of Total Phenolics

Estimation of total phenol was carried out using the method described by [10] as reported by [11]. The assay involved pipetting 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml garlic acid solution (1 mg/ml) in triplicate. The volumes were adjusted to 1.0 ml with distilled water. To each of the test tubes was added 1.5 ml Folin Ciocalteu’s reagent (1:10). The reaction mixture was incubated at room temperature for 15 minutes. To the mixture was added 1.5 ml of 7% (w/v) Na$_2$CO$_3$ solution. The reaction mixture was further incubated for 1 hr 30 min. The absorbance was read at 725 nm against the blank containing all reagents except the standard, garlic acid. The absorbance was plotted against the concentration to produce the standard curve. The latex (0.1 ml of 5 mg/ml) was pipetted into clean dry test tubes in triplicate and treated as standard. The concentration of phenolics from the latex was interpolated from the standard graph.

2.4.2. Estimation of Total Flavonoids

The concentration of flavonoids in *E. graminea* latex was estimated according to the procedure of [12]. The standard curve for the estimation of total flavonoids was prepared by pipetting 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of 1 mg/ml rutin (standard) into clean dry test tube. The volume was made up to 5.0 ml with distilled water. To each of the test tubes was added 0.3 ml of 5% (w/v) NaNO$_2$, 0.3 ml of 10% (w/v) AlCl$_3$ and 4 ml of 4% (w/v) NaOH. The reaction mixture was incubated at room temperature for 15 minutes. Absorbance taken at 500 nm was plotted against the concentration to give the standard curve.

The latex (0.1 g) was dissolved in ethanol (10 ml, 70% (v/v)). The working solution (0.5 ml) was pipetted into clean dry test tubes in triplicate and treated as standard. The concentration of flavonoids from the latex was interpolated from the standard graph.

2.4.3. Ferric Reducing Antioxidant Power Assay

The reducing power of the latex and the standard drug was determined according to the method of [13]. Different concentrations of *E. graminea* latex (0.1 mg/ml, 0.5 mg/ml and 1.0 mg/ml in distilled water) were mixed with 1.25 ml of phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (1.25 ml, 1%). The mixture was incubated at 50°C for 30 minutes and allowed to cool. To the mixture was added 10% (w/v) trichloroacetic acid, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (0.625 ml) was collected and mixed with 0.625 ml distilled water and FeCl$_3$ (0.125 ml, 0.1%). The absorbance of the mixture was measured at 700 nm against water blank. Ascorbic acid (Vitamin C) was used as a standard to compare the reducing power of the latex.

2.4.4. DPPH Radical Assay

The method described by [14] was used to determine the antioxidant activity of the latex. The latex scavenging activity of the stable DPPH free radical was determined at varying concentrations of 50, 100, 150, 200, 250, 300 µg/mL. Distilled water (500 µL) was pipetted (in triplicate) into clean dried test tubes followed by the addition of 0.0, 75, 150, 225, 300, 375, and 450 µL of the latex based on the varied concentrations, then 1500, 1425, 1350, 1275, 1200, 1125 and 1050 µL of DPPH solution.
was added to the test tubes respectively and mixed thoroughly. The reaction mixture was incubated in the dark for 30 minutes. The absorbance was taken at 517 nm and the percentage inhibition ability was calculated using the expression below.

\[
\text{Percentage Inhibition} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100.
\]

2.5. Evaluation of the Anti-Inflammatory Activity of E. Graminea Latex

2.5.1. Membrane Stabilization Assay

2.5.1.1. Preparation of red blood cell

The bovine red blood cell was prepared according to the method of [15]. The fresh blood sample was transferred into clean centrifuge tubes and followed by centrifugation at 3000 rpm on a Bench Centrifuge Model 90-2 (Searchtech Instrument England, UK.) for 10 min at room temperature. The supernatant was carefully removed and the packed red blood cell was washed again in fresh normal saline [0.85% (w/v) NaCl]. The process of washing and centrifugation was repeated until the supernatant was cleared (colourless). The bovine erythrocyte [2% (v/v)] was prepared by diluting 2 ml of packed cell with normal saline to 100 ml. It was kept undisturbed at 4°C in the refrigerator.

2.5.1.2. Red Blood Cell Membrane Stability Assay

The membrane stabilizing potentials of the latex was carried out according to the procedure earlier reported by [16]. Typically, varying concentrations (0, 100, 200, 300, 400 and 500 µg/ml) of the latex was pipetted into clean dry test tubes and adjusted to 1.5 ml with normal saline. To the reaction mixtures were added 0.5 ml hyposaline dry test tubes and adjusted to 1.5 ml with normal saline.

400 and 500 µg/ml) of the latex was pipetted into clean [16]. Typically, varying concentrations (0, 100, 200, 300, 400 and 500 µg/ml) of the latex was pipetted into clean centrifuge tubes and followed by centrifugation at 3000 rpm on a Bench Centrifuge Model 90-2 (Searchtech Instrument England, UK.) for 10 min at room temperature. The supernatant was carefully removed and the packed red blood cell was washed again in fresh normal saline [0.85% (w/v) NaCl]. The process of washing and centrifugation was repeated until the supernatant was cleared (colourless). The bovine erythrocyte [2% (v/v)] was prepared by diluting 2 ml of packed cell with normal saline to 100 ml. It was kept undisturbed at 4°C in the refrigerator.

The reaction mixtures were then incubated at 56°C for 30 min., cooled and centrifuged at 3000 rpm for 10 min. The supernatants were carefully decanted and absorbance was read at 560 nm against the reagent blank. The percentage membrane stability was calculated as:

\[
\text{Percentage Membrane Stability} = 100 - \frac{(\text{Abs}_{\text{blood control}} - \text{Abs}_{\text{drug}})}{\text{Abs}_{\text{blood control}}} \times 100.
\]

2.6. Evaluation of Wound Healing Activity of E. graminea Latex

2.6.1. Experimental Animals

Thirty-six wistar rats weighing between 120-150g of either sex were purchased at the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. They were acclimatised for two weeks in the Animal House, Department of Biochemistry, Adeleke University, Ede, Osun State, Nigeria. The rats were divided into three groups (separately for each model) of six rats each. Animals were provided with standard rodent pellet diet and water ad libitum. Principles of laboratory animal care guidelines were followed [17].

2.6.2. Incision Wound Model

Incision wound model was carried out according to the procedure described by [18]. In this model, cuts were made in the skin of the animal after giving them anaesthesia with ketamine (0.5 mL). Two para-vertebral long incision of 6 cm length was made through the skin and cutaneous muscles at distance about 1.5 cm from the midline on each side of the depilated back of the rats. After the skin incision, the parted skin kept together was stitched at 1 cm intervals continuously and tightly using suture thread (No. 000) and a curved needle (No. 11). When the wounds were cured thoroughly, the sutures were removed on day 7 and tensile strength of the healed wound was measured on day 10 by continuous and constant water flow technique.

2.6.3. Measurement of Tensile Strength

The sutures were removed on 7th post wounding day and the wound breaking strength of the wounds was measured on the 10th day according to the continuous constant water flow technique as described by [19]. Two Allis forceps were firmly applied onto the line facing each other. One of the forceps was fixed, while the other was connected to a freely suspended light weight polypropylene graduated container through a string run over to a pulley. Distilled water was continuously poured slowly and steadily into the container. A gradual increase in weight was transmitted to the wound site pulling apart the wound edges as at when the wound just opened up, the water was stopped and the weight was noted [18]. The percentage increase in wound breaking strength was calculated as follows:

\[
\text{Percentage increase in wound breaking strength} = \left( \frac{W_{\text{WT}} - W_{\text{WC}}}{W_{\text{WT}}} \right) \times 100,
\]

where WT and WC were WBS values of test and control groups respectively.

2.6.4. Dead Space Wound Model

The dead space wound model was carried out based on the procedure described by [20]. Rats were anesthetized with ketamine and 1 cm incision was made on dorsolumbar part of the back. Two polypropylene tubes (0.5×2.5 cm 2 each) were placed in the dead space of lumbar region of rat on each side, and wounds were closed with a suture material.

2.6.5. Grouping and Treatment of Animals

After the surgical wounds, all rats were randomly divided into three groups: A, B and C respectively. Group A: animals were treated with E. graminea latex dissolved in distilled water animals; Group B: animals were treated with an ointment, gentamycin cream (antibiotic drug) and served as the reference standard group; Group C: animals
were without treatment and served as the control group. The latex and the ointment were topically applied to the wound of the animals respectively. All rats were monitored daily for 15 days for any wound fluid, evidence of infection or other abnormalities.

2.6.7. Preparation of Granulation Tissue Homogenate
Granulation tissue homogenate was prepared as described by [21]. On the 15th day, the rats were euthanized via cervical dislocation. Thereafter, the implanted tubes were removed cautiously through incision on the animals’ skin, and weighed. Granulation tissue homogenates (10% w/v) were prepared by cutting granulation tissue (1 g) into bits and homogenized in freshly prepared phosphate buffer (pH 6.8, 100 mM). The homogenate was centrifuged at 3000 rpm with Bench Centrifuge Model 90-2 (Searchtech Instrument England, UK.) for 10 min. The supernatant was carefully transferred into clean vial bottles and kept frozen at -4°C for further biochemical assays.

2.6.8. Estimation of Wet Granulation Tissue Free Radicals

2.6.8.1. Determination of Nitric Oxide (NO) Concentration

Nitric Oxide assay was carried out using the Griess reagent [22]. The homogenized granulation tissue (500 μl) was pipetted into clean and dry test tubes and TCA (50 μl) was added to it. The resulting mixture was centrifuged for 10 min at 4000 rpm and the clear supernatant was transferred to a clean tube. The supernatant (100 μl) was pipetted into clean, labelled Eppendorf tubes and sulphanilamide (0.1 ml, 1%) in 5% phosphoric acid was added to it. The reaction mixture was incubated at room temperature for 10 min, followed by addition of 0.5 ml TCA (0.5 ml, 24%) and adrenaline. The reaction mixture was further incubated for 10 min at 60°C and the absorbance of the chromophore formed was measured at 546 nm. The standard curve was prepared using sodium nitrite (100 μM). The amount of NO in the sample was interpolated from the standard curve.

2.6.8.2. Determination of Malondialdehyde (MDA) Concentration

Total amount of lipid peroxidation product (malondialdehyde) in the homogenate was estimated according to the method described by [23]. The homogenate (0.5 ml) was pipetted into clean and dry test tubes, phosphate buffer (0.5 ml, 0.1 M, pH 8.0) and TCA (0.5 ml, 24%) were added to it respectively. The resulting mixture was incubated at room temperature for 10 min followed by centrifugation at 2000 rpm for 20 min. and the supernatant was collected. TBA (0.25 ml, 0.33%) in 20% acetic acid was added to 1 ml of supernatant and the resulting mixture was boiled at 95°C for 1 hr. The resulting pink color product was allowed to cool and absorbance was read at 532 nm. The results were expressed as mM TBARS/min/mg tissue at 37°C.

The concentration of malondialdehyde was calculated using this expression:

\[
\text{Malondialdehyde (mM TBARS/min/mg tissue)} = \frac{\text{Abs sample}}{\text{X DFlX}} \\
\text{Where I = light path, } \\
\text{X = Extinction coefficient of MDA at 532nm = 1.52 x 10^5 M}^{-1}\text{cm}^{-1} \\
\text{DF = Dilution factor}
\]

2.6.9. Estimation of Antioxidant Parameters in Wet Granulation Tissue

2.6.9.1. Estimation of Reduced Glutathione (GSH) Activity

Reduced glutathione (GSH) was measured by the method of [24]. Standard calibration curve was prepared using different concentrations of the standard GSH (0, 2, 4, 6, 8, 10 μg/ml). Ellman’s reagent (0.5 ml, 10 mM) and phosphate buffer (2 ml, 0.2 M, pH 8.0) were added to the different concentrations of the standard and the yellow colour developed was read at 412 nm. The homogenate (1 ml) was pipetted and treated as standard. The blank contained phosphate buffer only. The amount of GSH was expressed as mg/100g tissue.

2.6.9.2. Estimation of Superoxide Dismutase (SOD) Activity

The method described by [25] was used to assay for the activity of superoxide dismutase. To 50 μl of the homogenate, 75 mM of Tris-HCl buffer (pH 8.2), 30 mM EDTA and 2 mM of pyrogallol were added. An increase in absorbance was recorded at 420 nm for 3 min by spectrophotometer. One unit of enzyme activity is 50% inhibition of the rate of autooxidation of pyrogallol as determined by change in absorbance/min at 420 nm. The activity of SOD is expressed was expressed as units/mg protein.

\[
\text{Increase in absorbance} = \frac{A_3 - A_0}{2.5}
\]

Where A0 = absorbance after 30 seconds
A3 = absorbance after 150 seconds

% inhibition

\[=100 - 100 \times \left(\frac{\text{increase in absorbance of substrate}}{\text{increase in absorbance of blank}}\right)\]

One unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline.

2.6.9.3. Estimation of Catalase (CAT) Activity

The assay of CAT activity was carried out according to the method [26]. Typically, the homogenate (50 μl) in duplicate was added to a cuvette containing 450 μl of phosphate buffer (0.1 M, pH 7.4) and H2O2 (500 μl, 20 mM). CAT activity was measured at 240 nm for 60 sec (15, 30, 45, 60 sec.) against the reagent blank. The blank contained distilled water instead of the homogenate. The catalase activity was calculated using the expression:
Catalase activity (U/mg protein) = \frac{\Delta A_{\text{min}} \times d \times TV}{SV \times 0.0436} \text{ mg protein/mL.}

One unit of activity of catalase is equal to 1 mmol of H₂O₂ degraded per minute and expressed as units per milligram of protein.

2.6.10. Estimation of Connective Tissue Parameters

Approximately 250 mg of wet tissue was dried at 50°C for 24 hr. It was weighed and kept in glass stoppered test tubes. To each test tube containing 40 mg of dried granulation tissue was HCL (1 ml, 6N) added. The tubes were kept on boiling water bath for 24 hr (12 hr each day for two days) for hydrolysis. The hydrolysate was then cooled and excess of acid was neutralized by 10 N NaOH using phenolphthalein as indicator. The volume of neutral hydrolysate was diluted to a concentration of 20 mg/ml with distilled water. The final hydrolysate was used for the estimation of hexosamine and hexuronic acid following the standard curve prepared using the proper substrate.

2.6.10.1. Determination of Concentration of Hexosamine (HXA)

The method of [27] was used to determine the concentration of hexosamine. The hydrolysed fraction (0.05 ml) was diluted to 0.5 ml with distilled water. Acetyl acetone reagent (0.5 ml) was added to the diluted sample and heated in boiling water bath for 20 min and then cooled under tap water. Ethanol (95%, 1.5 ml) was added, followed by an addition of 0.5 ml of Ehrlich’s reagent and the reaction mixture was allowed for 30 min. Color intensity was measured at 530 nm against the blank. Hexosamine content of the samples was determined from the standard curve prepared with D (+) glucurono-6, 3-lactone, from 5 μg/0.5 ml to 40 μg/0.5 ml using 100 μg/mL working solution.

2.7. Statistical Analysis

Experimental data were expressed as mean ± standard error of mean (SEM). Statistical significance of differences among groups were evaluated using one-way analysis of variance (ANOVA) followed by Dunnett’s method of multiple comparisons using Graph Pad prism 5.0 Software. Data were considered significant at p < 0.05.

3. Results

3.1. E. graminea Latex Phytoconstituents

Phytochemical screening of EGL revealed the presence of alkaloids, flavonoids, cardiac glycosides, saponins and triterpenes (Table 1).

The total flavonoids and total phenolic contents were 6.400 ± 0.001 (μg/g RE) and 8.504 ± 0.047 (μg/g GAE) respectively (Table 2).

Table 1. Phytoconstituents of E. graminea Latex

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>result</th>
<th>Phytochemicals</th>
<th>result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>C. glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatannin</td>
<td>-</td>
<td>Steroids</td>
<td>-</td>
</tr>
<tr>
<td>Tannin</td>
<td>-</td>
<td>Triterpenes</td>
<td>+</td>
</tr>
<tr>
<td>Xanthoproteins</td>
<td>-</td>
<td>Anthraquinone</td>
<td>-</td>
</tr>
</tbody>
</table>

+ positive, - negative, C-Cardiac.

Table 2. E. graminea Latex Total Flavonoids and Phenolics Concentrations

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total flavonoid (μg/g RE)</th>
<th>Total phenolic (μg/g GAE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. graminea Latex (µg/mg)</td>
<td>6.400 ± 0.001</td>
<td>8.504±0.047</td>
</tr>
</tbody>
</table>

Each value represented the mean ± SEM, in triplicates (n=3) R - Rutin; G- Garlic acid E- Equivalent.

3.2. Antioxidant Capacity of E. graminea

The latex of E. graminea inhibited DPPH in dose dependent manner (Figure 1).

It also exhibited mild ferric reducing (IC₅₀ - 3.304 ± 0.245 μg/ml) compared to ascorbic acid (IC₅₀ -1.011 ± 0.245 μg/ml) (Table 3).
Table 3. Ferric reducing antioxidant potential of *E. graminea* latex

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC₅₀ (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGL</td>
<td>3.304 ± 0.831</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>1.011 ± 0.245</td>
</tr>
</tbody>
</table>

3.3. Membrane Stability Activity of *E. graminea* and Diclofenac on Bovine Red Blood Cell

*E. graminea* latex exhibited minimum percentage stability of 98.98%, 96.95%, 98.95%, 98.90% at 0.5, 1.0, 1.5 and 2.0 mg/mL respectively while the minimum percentage stability of diclofenac at 0.5, 1.0, 1.5 and 2.0 mg/mL were 18.94%, 18.35%, 74.00%, 27.06% respectively. The maximum percentage stability at 0.5, 1.0, 1.5 and 2.0 mg/mL for *E. graminea* latex were 99.05%, 98.44%, 99.10%, 98.99% respectively while the maximum percentage stability for the standard (diclofenac) at the same concentrations were 76.35%, 80.94%, 86.86%, 89.06% respectively. At 0.5 and 1.5 mg/mL, *E. graminea* latex stabilized the stressed erythrocyte membrane maximally at 99.05% and 99.10% better than 2 mg/mL diclofenac with maximum stability at 89.06%.

3.4. Effect of *E. graminea* and Gentamycin in Incision Wound Model

Control group rats showed wound breaking strength (WBS) value of 680.00 ± 0.00 g on 10th post wound day. EGL treated rats showed significant increase in WBS by 13.60% and the effect on WBS was comparable with Gentamycin ointment treated rats which showed 20.96% significant increase.

Table 4. Effects of *E. graminea* latex and Gentamycin on Wound Breaking Strength (WBS)

<table>
<thead>
<tr>
<th>Sample</th>
<th>WBS (g)</th>
<th>(PIWBS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound + EGL</td>
<td>772.50 ± 20.96&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>13.60</td>
</tr>
<tr>
<td>Wound + G</td>
<td>822.50 ± 13.60&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>20.96</td>
</tr>
<tr>
<td>Wound only</td>
<td>680.00 ± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Values were represented as Mean ± SEM from 5 animals in each group (n = 5).

Superscript a, b and c indicate significant (p < 0.05) difference from control, wound + Gentamycin (G) and wound + *E. graminea* Latex (EGL) respectively at 95% C.I.

Percentage increase in wound Breaking strength (PIWBS) = \(\frac{WT}{WC} - 1\)×100,

where WT and WC were WBS values of test and control groups respectively.

3.5. Effects of *E. graminea* Latex and Gentamycin on Hexoxamine and Hexuronic Acid Concentrations

There was significant (p < 0.05) elevation in hexoxamine and hexuronic acid concentrations in both EGL and gentamycin treated rats when compared to the control rats.

Table 5. Effects of *E. graminea* latex and Gentamycin on Hexoxamine and Hexuronic acid Concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>Hexoxamine (μg/mL)</th>
<th>Hexuronic acid (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound+EGL</td>
<td>85.21 ± 18.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.16 ± 2.49&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wound + G</td>
<td>98.61 ± 3.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.59 ± 0.80&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wound only</td>
<td>70.07 ± 12.89</td>
<td>1.45 ± 0.48</td>
</tr>
</tbody>
</table>

Values were represented as Mean ± SEM from 5 animals in each group (n = 5).
Superscript a, b and c indicate significant (p < 0.05) difference from control, wound + Gentamycin (G) and wound + E. graminea Latex (EGL) respectively at 95% C.I.

3.6. Effects of *E. graminea* on Wet Granulation Tissue Antioxidants and Free Radicals

There was significant (p < 0.05) increase in the level of superoxide dismutase (SOD) and reduced glutathione (GSH) in the EGL and Gentamycin treated rats when compared with the control. Gentamycin significantly (p < 0.05) increased the GSH concentration, when compared with the EGL treatment. There was no significant (p < 0.05) difference in the level of NO and catalase activity rats treated with *E. graminea* latex and Gentamycin were compared with the control rats. However, EGL treatment significantly (p < 0.05) decreased the lipid peroxidation end product (MDA) when compared with the control group.

| Table 6. Effects of *E. graminea* Latex and Gentamycin on Antioxidants and Free Radicals |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Group                                        | SOD (mU/mg)     | CAT (mg/100 mg) | GSH (mU/mg)     | MDA (µM)        |
| Wound+EGL                                    | 0.63± 0.15      | 0.0058          | 6.52± 0.25      | 2.32± 0.88      |
| Wound+ G                                     | 0.59± 0.18      | 0.0026± 0.0049  | 10.45± 0.29     | 2.96± 1.99      |
| Wound (Control)                              | 0.19± 0.01      | 0.0069          | 8.82± 0.26      | 3.16± 1.22      |

Values were represented as Mean ± SEM from 5 animals in each group (n = 5).

The overall role of antioxidants appears to be significant in the successful treatment and management of wounds. Antioxidants reduce these adverse effects of wounds by removing products of inflammation. They counter the excess proteases and ROS often formed by neutrophil accumulation in the injured site and protect the tissue cells and the extracellular matrix [42]. At high concentrations, ROS can induce severe tissue damage and even lead to neoplastic transformation decreasing the healing process by damages in cellular membranes, DNA, proteins and lipids [43].

4. Discussion

Bioactive compounds of plant origin have beneficial effect on wound care and management by accelerating the rate of epithelisation with least scarring of skin tissue [4]. Wound healing process consists of re-epithelization and reconstruction of tissue matrix collagen. It is an innate immunological activity happening on its own, but factors like tissue ROS generation and inflammation not only hindered the healing process but also increased the chances of secondary infection [29,30]. Medicinal plants that have antioxidant and anti-inflammatory properties have been reported to reduce the wound healing process [31,32].

The result of this study revealed that *E. graminea* latex possesses appreciable concentrations of phenolics and flavonoids content. Phenolic compounds, especially flavonoids and phenols have been shown to possess significant antioxidant activity which can help in the treatment of wound [33,34,35,36]. Flavonoids and phenolics are powerful antioxidants against free radicals and are described as free radical scavengers [37,38].

In this study, DPPH radical scavenging assay and the ferric reducing antioxidant potential assay were used to investigate the antioxidant activity of EGL. The DPPH radical scavenging assay is a rapid, simple and convenient method of investigating the ability of phenolic compounds in extract to act as electron donor [39]. Numerous plant constituents have been demonstrated to exhibit free radical scavenging or antioxidant activity [11]. The *in vitro* DPPH radical scavenging assay revealed that EGL has a potent antioxidant activity. Evident from this study, a linear relationship was observed between the decrease in absorbance of DPPH and different concentrations. The increased concentration of the latex showed increased DPPH radical scavenging activity.

Ferric reducing antioxidant power (FRAP) is used to assess the metal (iron exclusively) ions binding ability [40]. *E. graminea* latex exhibited mild ferric reducing antioxidant activity of (IC50 value of 3.304 ± 0.831 mg/ml) comparable to the standard, ascorbic acid (IC50 value of 1.011 ± 0.245 mg/ml). IC50 value is defined as the concentration of substrate that causes 50% loss of free radicals activity and was calculated by linear regression of plots of the percentage of antiradical activity against the concentration of the tested plant latex. The lower the IC50 value, the higher the antioxidant potential. The antioxidant properties of the latex may be attributed to the presence of bioactive compounds like flavonoids and alkannoids with antioxidant potential [37,38].

Reactive oxygen species (ROS) is produced in high quantities at the site of wound as a defense mechanism against invading bacteria [41]. However, the presence of increased numbers of neutrophils and ROS overwhelm the antiprotease substances that normally protect the tissue cells and the extracellular matrix [42]. At high concentrations, ROS can induce severe tissue damage and even lead to neoplastic transformation decreasing the healing process by damages in cellular membranes, DNA, proteins and lipids [43].
strength had been reported to be acquired from both re-modeling of collagen and formation of stable intra and inter molecular crosslinks [48,49]. The wound healing accelerating potential of the latex may be attributed to the presence of bioactive compounds such as flavonoids, alkaloids, saponins, c. glycosides and triterpenes present in the plant [50].

The enhanced capacity of wound healing accelerating potential with the plant was on the basis of anti-inflammatory effect of the plant [51]. This was demonstrated in vitro by the membrane stabilizing potential of the E. graminea latex. The latex showed highest stability of the stressed red cell membrane at higher percentage than the standard, anti-inflammatory drug (diclofenac).

The second model, dead space wound model, is mainly for evaluating the efficacy of drugs on the connective tissue markers which include hexosamine and hexuronic acid. Implantation of polypropylene tubes causes inflammatory response and modulates the release of inflammatory mediators which finally results in tissue proliferation and granulation tissue formation [52]. Hexosamine and hexuronic acids (glycosaminoglycans) are matrix molecules which act as ground substratum for the synthesis of new extracellular matrix [53]. Secondary metabolites like tannins, steroids and saponins are known to have antimicrobial and anti-inflammatory effect that can be responsible for the treatment of gastrointestinal infection, swellings and wounds [54]. In this study, the concentrations of hexuronic and hexosamine were significantly increased (P<0.05) in wounds treated with EGL when compared with the control indicated the stabilization of granulation tissue.

Furthermore, it was observed that treatment of wound with EGL caused a significant increase in wet granulation tissue GSH and SOD when compared to the control animals. Overproduction of reactive oxygen species ROS results in oxidative stress thereby causing cytotoxicity and delayed in wound healing [55]. Intercellular and extracellular antioxidants are thus important to protect tissues from oxidative injury [56]. From the study, decrease in wet granulation tissue NO (though not significant) and MDA concentrations in EGL treated wound was observed compared to control. Free radicals and ROS are constantly formed by the human body; ROS mediate cell damage. Intercellular and extracellular antioxidants are thus important to protect tissues from oxidative injury [57]. Superoxide dismutase (SOD) converts superoxide to hydrogen peroxide (H₂O₂), which is then converted into water by catalase in lysosomes or by glutathione peroxidase (GPx) in mitochondria [56]. GSH reduces H₂O₂ and lipid peroxides via reactions catalyzed by GSH peroxidase [58]. Topical application of EGL significantly (P<0.05) enhanced the activity of SOD and GSH concentration but no significant effect on catalase. These prevented the harmful effects of free radicals generated in rats. Superoxide and hydroxyl radicals are vital mediators of oxidative stress that contribute to clinical disorders. Therefore, any natural or synthetic element with antioxidant properties can totally or partially reduce the harm caused by free radicals [59].

This suggests that EGL at the concentration applied probably possess very little or no influence to restrain free radicals generated stress.

5. Conclusion

This study revealed that E. graminea latex possessed significant antioxidant and anti-inflammatory properties as well as wound healing accelerating potential confirmed by stabilized collagen fibers.

It could therefore be surmised that the therapeutic principles contained in E. graminea latex could possibly be employed in boosting human defense system as well as treatment of wound.

References

American Journal of Biomedical Research


Grisham, M. B., Johnson, M. D., Gautreaux, R. D., Methods, A Comp. to Meth. in Enz., 7: 84-90. 1995.


