Indian J. Nat. Prod, 2022; 36(1):44-51
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
http://www.ijnponline.com

Original Article

Anti-inflammatory and Wound Healing Activity of Gymnospermous Plant *Cycas revoluta*

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ABSTRACT

Background: Wounds are a serious worldwide health problem that place significant economic, financial, and social strain on health care organizations, professionals, patients, and their families. **Aim:** To investigate the anti- inflammatory and wound healing activity of ethanolic extract of leaves *Cycas revoluta*. **Materials and Methods:** In albino rats, the anti- inflammatory and wound healing activity was explored using complete excision, incision wound model and paw edema induced wound model. The wound healing and anti-inflammatory activity were then compared to that of other standard medications available. **Results:** In both models investigated, different dosages of CREE (200 mg/kg and 400 mg/kg) significantly improved wound healing activity. When compared to the control group of mice, animals treated with CREE had a higher rate of wound contraction ($p<0.001$), a shorter period for epithelialization ($p<0.01$), a higher skin breaking strength ($p<0.001$), and a higher hydroxyproline content. In addition, histopathological investigations of the CREE-treated groups demonstrated better wound healing. **Conclusion:** The study reveals the wound healing and anti-inflammatory activity of ethanolic extracts (95%) of leaves part of *Cycas revoluta* in complete wound healing and anti-inflammation model. The extracts seem promising for the development of phytomedicine for wound healing and anti-inflammatory and study provides future research in screening the extract constituents responsible for the wound healing and anti-inflammatory activity.

Keywords: *Cycas revoluta,* Wound healing, Anti-inflammatory, Ethanolic extract (95%).

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DOI : 10.5530/ijnp.2022.1.7

INTRODUCTION

A wound or tissue rupture is described as a rupture of the normal anatomical connections of tissues caused by trauma or injury. The damage may be deliberate, such as a surgical incision, or it may be unintentional as a result of trauma.[1] When tissue is damaged, the normal wound healing signalling cascade begins. The process of restoring damage to the skin and other soft tissues is known as wound healing. When an injury occurs, an inflammatory response occurs, and the tissue underneath the dermis begins to generate more collagen. Later, the epithelial tissue of the skin regenerates.[2] Wound healing is widely considered to be a physiologic mechanism mediated by the immune system.[3]

Wound healing is a complex process through which the skin heals itself after injuries.[4] Healing of wounds or tissue rupture begins at the time of any physical harm caused by cutting or firing and can last for various amounts of time depending on the degree of the damage. The process should be broadly classified into 3 stages: inflammatory, proliferative, and remodeling stage which ultimately defines the strength and appearance of the healed tissue.[5] Wound healing or rupture tissue is a complicated set of interconnected processes mediated by a wide variety of chemically coordinated cellular events as well as hormonal events. Chemical mediators such as growth factors, cytokines, and chemokines are combined to co-ordinate the wound healing process, allowing the body to repair injured tissue and restore skin integrity.[6]

Wound rupture encompasses cellular, molecular, biochemical, and physiological processes that result in connective tissue healing and the development of fibrous scars, as well as the restoration of tissue and skin anatomical continuity and functional status.[7] The wound-healing process is divided into four interconnected and overlapping stages: hemostasis, inflammation, and tissue remodeling.[8] There are phases, and their biophysiological activities occur in the correct order, at a specific time, and continue for a specified length of time at an optimal intensity.[9]

A number of studies have been conducted to assess the activity of traditionally used herbs in the treatment of skin disorders, including wound injuries e.g., *Rafflesia hasseltii,*[10] *Morinda citrifolia,*[11] *Momordica charantia*[12] and *Limonia acidissima* Linn.[13]

The objective of this study was the evaluation of anti-inflammatory and wound healing properties of *Cycas revoluta* leaf extract. The plant *Cycas revoluta* belongs to family Cycadaceae. It is also commonly known as Japanese Sago Palm, Sago palm, King Sago Palm.

MATERIALS AND METHODS

Drugs and Chemicals

Soframycin and indomethacin were purchased from Sanofi pharmaceuticals and other chemicals such as formalin, petroleum ether, ketamine HCl and other essential chemicals were purchased from Sigma/Aldrich Chemicals Co. Analytical grade chemicals and reagents were utilised throughout the experiment.

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Figure 1: *Cycas revoluta*.

Collection and Authentication of Plant Materials

An ornamental plant as shown in Figure 1, cultivated in semi-arid areas of India the fresh leaves. The fresh leaves of *Cycas revoluta* was obtained from the local drug market of Allahabad, Uttar Pradesh in month of October 2017. Plant material was identified and authenticated by Dr. D. K. Chauhan, Taxonomist, University of Allahabad, Allahabad, and a specimen voucher (AU/BOT/AUT/2016/11), deposited in the Department of Botany, University of Allahabad, Allahabad.

Preparation of Plant Extract

Shade-dried *Cycas revoluta* leaves were crushed to a coarse powder and passed through sieve number 20 to preserve consistency, then pulverized to a coarse powder and passed through sieve number 20 to maintain uniformity. To remove fatty components, coarsely dried powder of the leaves was first defatted with petroleum ether (60–80 C) for 48 hr. The extract was collected, filtered through muslin cloth followed by filter paper (Whatman No. 1), and concentrated in an EYELA rotary evaporator at reduced pressure for 48 hr with 500ml ethanol (60–70 C) (Sigma-Aldrich, USA). For additional investigation, the dried extract was kept at 20°C. The extract yield was determined as a percentage yield.

Phytochemical Screening

Cycas revoluta ethanolic leaves extract (CREE) was subjected to various phytochemical screening tests for the identification of the phytoconstituents present using standard procedures.[14]

Experimental Animals

Healthy albino rats weighing between 150 and 200 gm of either sex and of approximately the same age were used for the present study. Animals were procured from the authorized animal house of United Institute of Pharmacy's animal house in Naini, Prayagraj, Uttar Pradesh, India. The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at 25 ± 2°C, relative humidity 44–56%, and light and dark cycle of 12:12 hr and fed with standard diet and water *ad libitum* during the study. The ethical permission for this study was obtained from the United Institute of Pharmacy in Naini, Prayagraj, Uttar Pradesh, India. All experiments were performed in the morning according to the current guidelines for investigation of experimental pain in conscious animals. REG. No.-1451/PO/E/11/CPCSEA dated 04/05/2011 under rule 3 of the breeding of and experiments on animals (control and supervision) rules.

Acute Oral Toxicity

Healthy Albino Wister rats (200 - 250gm) of either sex were selected. These animals were starved and orally fed the extracts of *Cycas revoluta* leaves in increasing dose level of 50, 100, 250, 500, 1000, 2000 and 5000 mg/kg of body weight. Rats were observed for 24 hr for any lethality.

Anti-inflammatory Activity

Formalin-induced anti-inflammatory model

Albino Wister rats (200-250gm) were used for the pharmacological study of drugs. The rats were divided into five groups of six rats in each group. Inflammation was produced in rats by injection of 0.1ml of 1% w/v formalin into the sub plantar region of left hind paw.[15,16] For pharmacological study following groups were divided. Group I administered distilled water, to serve as normal control animals. Group II administered formalin (0.1ml/kg), to serve as formalin control animals. Group III administered formalin (0.1ml/kg) and indomethacin (40mg/kg), as standard animals. Group IV (Test group) administered formalin (0.1ml/kg) and *Cycas revoluta* ethanolic extract at a dose of 200mg/kg, as test animals. Group V administered formalin (0.1ml/kg) and *Cycas revoluta* ethanolic extract at a dose of 400mg/kg as test animals. The paw volume was measured by plethysmometric apparatus, before formalin injection and after 30 min formalin injection. 30 and 60 min after drug administration rat paw oedema volume was measured of each and every group. The average paw swelling in the groups of extract treated rats were compared with control group and change in oedema was calculated.

Wound Healing Activity

For excision and incision wound model, animals were divided into four groups each consisting of six animals as follows: group I, left untreated and considered as control, group II, which served as standard and was treated with soframycin (25mg/kg), groups III and IV which were treated with 200mg/kg and 400mg/kg CREE, respectively. All the treatments were given twice daily.

Excision Method

Excision wound was created as per the method described;[17] four groups of animals each containing six rats were shaved on the dorsum portion using depilatory cream (Reckitt Benckiser, Inc., UK) and anesthetized using ketamine hydrochloride (50 mg/kg, i.p, body weight). An impression was made on shaved dorsal region and area of the wound to be created was marked. A full thickness excision wound with a circular area of 314 mm^2 was created along the marking using toothed forceps, a surgical blade, and pointed scissors. Rats were left undressed to the open environment. The different doses of extract and standard drug were applied twice daily from the day of the operation until the complete healing. In this model, wound contraction and epithelialization period were evaluated. Wound contraction was measured as percent contraction every 4th day after wound formation. At the end of the study, all the rats were anesthetized and from the healed wounds, specimen samples of tissue were collected from each rat, leaving a 5 mm margin of normal skin around the edges of the healed wound. Specimen tissues were stored in 10% formalin solution and used for histopathological and biochemical studies.[18]

Incision Wound Model

Incision wound was created according to the method described.[19] The animals were grouped and treated the same as in the excision wound

model. All rats were anesthetized using ketamine hydrochloride (50 mg/kg, i.p, body weight). Paravertebral incision of 6 cm length was made through the entire thickness of the shaved skin, on either side of the vertebral column of the rats with the help of a sharp scalpel. The wound was left undressed and animals were treated daily for 21 days. On the 21th day, all rats were anesthetized and sutures were removed and tensile strength of cured wound skin was measured.

Wound Healing Evaluation Parameters

Measurement of Wound Contraction and Epithelialization Period

Wounds of the first group of animals were topically treated with 0.2 ml of vehicle, gum acacia in normal saline (20 mg/ml), twice daily as placebo control group.[20] Wounds of the second group rats were topically treated with 0.2 ml of Intrasite gel twice daily as a reference standard control. Moreover, 0.2 ml of ethanol extract of *C. revolute* in vehicle was applied topically twice daily to the wound of third and fourth groups respectively. All animals were sacrificed on 21th day post-wounding surgery. The wound closure area of each animal was assessed by tracing the wound on days 3, 6, 9, 14 and 21 post-wounding surgery and the wound closure rate was expresses as the percentage of wound area compared with that on post-operative day by using transparency paper and a permanent marker under general anesthesia (a mixture of Ketamine and Xylazine) as described by Nayak and Pinto-Pereira (2006).[21] The wound areas recorded were measured using a graph paper. The percent wounds healing on these days are determined.[22]

In the excision wound model, wound area was measured by tracing the wound with the help of transparent sheet using millimeter-based graph paper on days 3, 6, 9, 14 and 21 for all groups. Wound contraction was measured every 3rd day until complete wound healing and represented as percentage of healing wound area.[23] Percentage of wound contraction was calculated taking the initial size of the wound as 100% using the following formula:

$$\% \text{ wound contraction} = \frac{(\text{Initial wound area} - \text{Specific day wound area})}{\text{Initial wound area}} \times 100$$

Epithelialization period was calculated as the number of days required for falling off the dead tissue remnants of the wound without any residual raw wound.[24]

Determination of Wound Healing (Wound Closure)

Skin wound areas were traced manually and calculated in square millimeters; the wound was observed daily until complete wound-healing enclosure completely occurs. The wound closure area of each animal was assessed by tracing the wound on days 3,6,9,14 and 21 post-wounding surgery and the wound closure rate was expressed as the percentage of wound area compared with that on post-operation day by using a transparent paper and a permanent marker under general anesthesia (Ketamine and Xylazil). The percent of wound healing on these days were determined.[25]

Measurement of Tensile Strength

The tensile strength of a healing skin wound indicates the degree of wound healing. It represents how much the healed tissue resists to breaking under tension and may identify the quality of healing tissue. The Tensile strength (skin breaking strength) of the 10-day old wound was measured. (Table 5) by this method. The rats were secured to the operating table, anesthetized by injecting ketamine hydrochloride (50 mg/kg, i.p, body weight) and a line was drawn on either side of the wound 3 mm away from the wound. Two allice forceps were firmly applied to the line facing each other. One of the forceps was fixed, while the other was connected to a freely suspended lightweight polypropylene graduated container through a string run over to a pulley. Water was allowed to flow from the reservoir slowly and steadily into the container. A gradual increase in weight was transmitted to the wound site pulling apart the wound edges. The moment the wound just opened up, the water flow was arrested and the volume of water collected in the container (approximately equal to its weight) was noted. Three readings were recorded for a given incision wound and the procedure was repeated on the wound on the contra lateral side. The mean reading was taken as the breaking strength for a given group in the incision model.[26]

Hydroxyproline Estimation

Excised wound tissues from all rats were analyzed for the estimation of hydroxyproline. Tissues were dried in a hot air oven at 60°C to constant weight and were hydrolyzed in 6 N HCl for 4 h at 130°C. The hydrolysates were then neutralized to pH 7.0 and were subjected to Chloramine-T oxidation for 20 min. After 5 min, the reaction was terminated by the addition of 0.4 M perchloric acid and developed color with Ehrlich reagent at 60°C. After thorough stirring the samples were analyzed at 557 nm in ultraviolet (Systronics-2203) spectrophotometer. The hydroxyproline content in the tissue samples was calculated using a standard curve of the pure L-hydroxyproline.[27]

Histopathological Study

The animals were sacrificed by cervical dislocation method after a dose of ketamine. The specimens of wound tissue were collected and preserved in glass vials containing 10% formalin solution for histological examination. Sections of wound tissue specimens (about 5 μm thickness) were prepared by microtomy and stained with hematoxylin and eosin (H&E) dye for histological examination. Incubation was done at 37°C under controlled condition for histopathological estimation. The histopathology was performed at United Diagnostic and Research, Allahabad.

Statistical Analysis

The results are expressed as mean ± standard error of mean (SEM). The statistical significance was analyzed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons Test employing statistical software, GraphPad, InStat 3. Differences between groups were considered significant at $P < 0.05$ levels.

RESULTS

Physicochemical Parameters Analysis

Physicochemical parameters of *Cycas revoluta* like loss on drying (2.8 %), percentage swelling index (0.6 %), total ash value (6.1 %), acid insoluble ash (0.8 %), water soluble ash (3.6 %), ethanol soluble extractive (6.8 %) and foaming index (4.5 %) were determined.

Fluorescence Analysis

The result of Fluorescence of crude powder of *Cycas revoluta* leaves shown in Table 1.

Phytochemical Analysis of *Cycas revoluta*

The result of phytochemical analysis of crude powder of *Cycas revoluta* leaves shown in Table 2.

Table 1: **Fluorescence analysis of *Cycas revoluta* leaves.**

Sl. No.	Reagents	UV Light (254nm)	UV Light (366nm)	Visible light
1.	Powder as such	Green	Brown	Greenish yellow
2.	Conc. Sulphuric acid	Black	Black	Black
3.	50% Sulphuric acid	Black	Blackish red	Greenish brown
4.	Conc. HCl	Black	Blackish red	Greenish black
5.	50% Conc.	Black	Blackish red	Light brown
6.	Conc. Nitric acid	Greenish yellow	Yellow	Blackish orange
7.	50% Conc. Nitric acid	Greenish yellow	Yellow	Blackish orange
8.	10% Sodium hydroxide	Greenish brown	Black	Blackish red
9.	5% Ferric chloride	Greenish brown	Black	Dark green
10.	5% Potassium hydroxide	Dark green	Brown	Greenish black
11.	With water	Dark brown	Light brown	Light brown
12.	Acetic acid	Greenish red	Light brown	Light brown

Table 2: **Phytochemical analysis of *Cycas revoluta* leaves.**

Sl. No.	Test Performed	Ethanolic extract of *Cycas revolute*
1.	**Alkaloids**	
	Mayer's reagent	+++
	Dragendorff's reagent	++
	Wagner's reagent	+++
	Hager's reagent	+
2.	**Saponins**	
	Froth test	–
3.	**Steroids**	
	Salkowasld test	--
	Liebermann's reagent	-
4.	**Carbohydrates**	
	Molisch's test	--
	Fehling's test	-
5.	**Anthraquinone Glycosides**	
	Borntrager's test	+
6.	**Cardiac Glycosides**	
	Legal test	++
	Keller killiani test	++
7.	**Tannins**	
	Lead acetate solution	---
	Ferric chloride solution	--
8.	**Proteins**	
	Xanthoprotein test	–
	Biuret test	–
9.	**Flavonoid test**	
	Ammonia test	+++
	Alkaline reagent test	+++
	Magnesium ribbon test	+++
10.	**Phenols**	
	$FeCl_3$ test	+++
	Ellagic acid test	-

Table 3: **Effect of ethanolic extract (95%) of *Cycas revoluta* in Percent inhibition of Anti-inflammatory activity.**

Treatment/ Percent Inhibition	1st hr	2nd hr	3rd hr	4th hr	6th hr
Normal control	1.1± 0.04	1.2± 0.03	1.15± 06	1.16± 08	1.14± 09
Control	2.19± 07	2.2± 0.001	2.4± 0.02	2.5± 0.02	2.21± 0.02
Indomethacin 40mg/kg i.p	1.23± 0.02**	1.25± 0.02	1.4± 0.03	1.3± 0.01	1.45**± 0.01
CREE 200mg/kg	1.8± 0.01	1.9± 0.04	1.8± 0.01	1.7± 0.03	1.8± 0.01
CREE 400mg/kg	1.4± 0.01	1.5± 0.02	1.2***± 0.02	1.25± 0.01	1.22± 0.02

The data was represented as Mean ±SD six rats per group. *$p < 0.05$, **$p < 0.01$ ***$p < 0.001$ inflammation when compared with positive control.

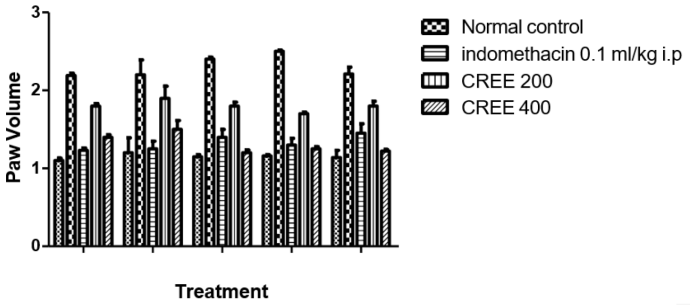

Figure 2: Effect of CREE in Percent inhibition in wound heal.

Table 4: **Effect of ethanolic extract (95%) of *Cycas revoluta* Percent wound contraction of excision model.**

Percent Wound Contraction	Day 3	Day 6	Day 9	Day 14	Day 21
Control	10.24± 0.02	30.58± 0.01	71.06± 0.01	86.6± 0.01	93.43± 0.02
Soframycin 25mg/kg	11.84± 0.01	34.21± 0.001	64.33± 0.02	78.95± 0.002	98***.28± 0.01
200mg/kg	14.22± 0.02	38.61± 0.01	78.49**± 0.01	86.96± 0.03	93.25± 0.03
400 mg/kg	16.07± 0.03	41.49± 0.02	78.49± 0.03	87.07± 0.01	96.97**± 0.01

The data was represented as Mean ±SD six rats per group. *$p < 0.05$, **$p < 0.01$ ***$p < 0.001$ when compared to wound healing when compared to positive control.

Percent Inhibition

Percent Wound Contraction

A significant difference in wound closure was observed between the four groups from day 3 onwards; in later days, the rate of wound closure in the treated group was much faster than that in the control group (Table 7). Complete wound closure was observed in the group treated with the *C. revoluta* leaf extract on day 3, 6, 9, 14, whereas it took about 21 days in the control group. Results are shown in Table 3 and 4.

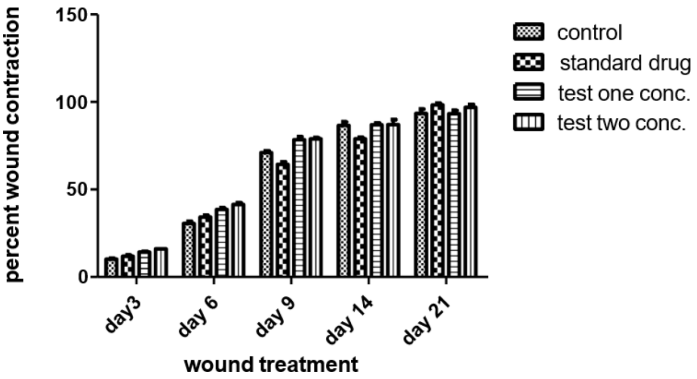

Figure 3: Effect of CREE in Percent wound contraction in wound healing.

Table 5: Effect of ethanolic extract (95%) of *Cycas revoluta* Tensile strength of incision wound model.

Tensile strength	Day 21
Control	63.94 ±0.01
Soframycin 25 mg/kg	325.50±0.002***
200 mg/kg	74.19±0.03
400 mg/kg	110.77±0.01**

The data was represented as Mean ±SD six rats per group. *p < 0.05, **p <0.01 ***p < 0.001 when compared to wound healing when compared to positive control.

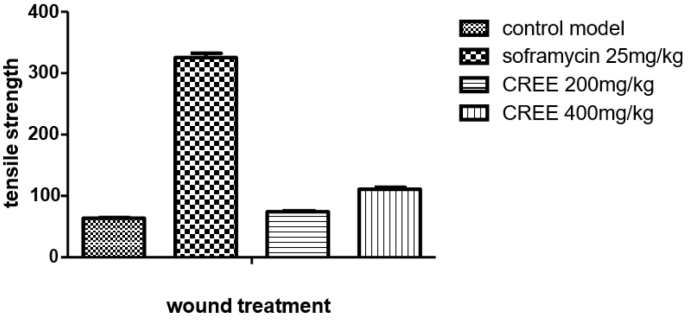

Figure 4: Effect of CREE in Tensile strength in wound healing.

Tensile Strength

The Tensile strength (skin breaking strength) of the 10-day old wound was measured. The rats were secured to the operating table and a line was drawn on either side of the wound 3 mm away from the wound.

Estimation of Hydroxyproline

Increased hydroxyproline content ultimately responsible for increasing the collagen level confirmed the increased viability or microcirculation of collagen fibrils around the wound area. The hydroxyproline level was found to be significantly elevated (p < 0.01) in treated group animals in a concentration dependent manner in comparison to control. The relative order for different groups in accordance to collagen stability or wound strength was at standard 25mg/kg soframycin > extract 400mg/kg > extract 200mg/kg > control.

Wound Closure

A significant difference in wound closure was observed between the four groups from day 3 onwards; in later days, the rate of wound closure in the

Table 6: Effect of ethanolic extract (95%) of *Cycas revoluta* in hydroxyproline assay.

Hydroxyproline assay	Day 21
Control	64.66±0.02
Soframycin 25 mg/kg	133.41**±0.001
Drug 200 mg/kg	252.44±0.01
Drug 400 mg/kg	303.68***±0.002

The data was represented as Mean ±SD six rats per group. *p < 0.05, **p <0.01 ***p < 0.001 when compared to wound healing when compared to positive control.

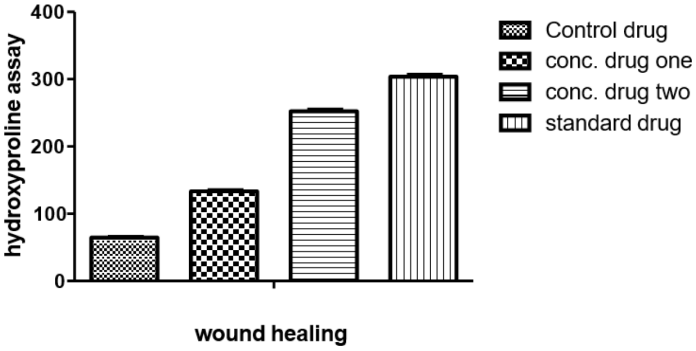

Figure 5: Effect of CREE in Hydroxyproline assay in wound healing.

Table 7: Effect of ethanolic extract (95%) of *Cycas revoluta* wound closure determination.

Wound Closure	Day 3	Day 6	Day 9	Day 14	Day 21
Control	4±0.01	3.88±0.02	3.78±0.002	3.55±0.001	3.43±0.04
Soframycin 25 mg/kg	4.16±0.002	3.88±0.02	3.61±0.03	3.35±0.02	2.91±0.001
Dose 200 mg/kg	4.21±0.02	3.96±0.01	3.63±0.02	3.2±0.01	2.23±0.01
Dose 400 mg/Kg	4.20±0.003	3.88±0.03	3.31±0.002	2.63±0.03	1.2±0.001

The data was represented as Mean ±SD six rats per group. *p < 0.05, **p <0.01 ***p < 0.001 when compared to wound healing when compared to positive control.

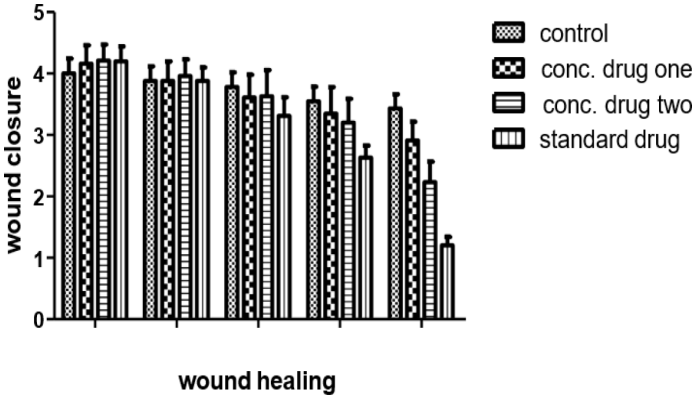

Figure 6: Effect of CREE in Wound contraction in wound healing.

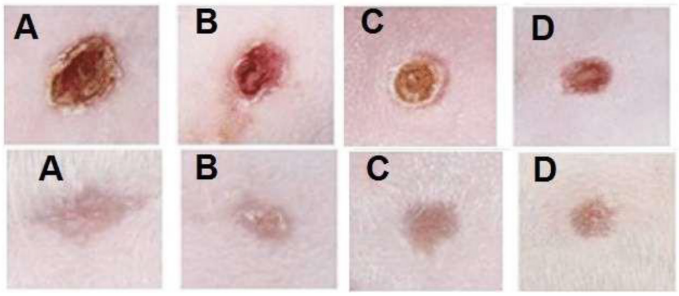

Figure 7: Excision model: A-control, B- Soframycin 25 mg/kg C- dose one 200 mg/kg D- dose two400 mg/kg.

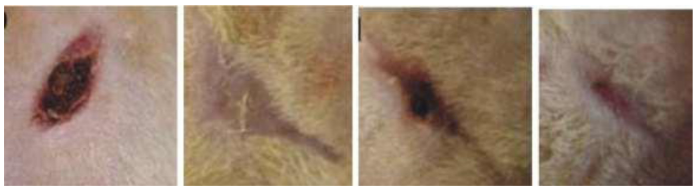

Figure 8: Incision model: A-control, B- Soframycin 25 mg/kg C- dose one 200 mg/kg D- dosetwo 400 mg/kg.

treated group was much faster than that in the control group. Complete wound closure was observed in the group treated with the *Cycas revoluta* leaf extract on day 6,9,14, whereas it took about 21 days.

Excision Wound Healing Activity of Cree

In the excision wound model we can saw the control groups rat were normal as compare to standard groups. And compare to control group, CREE dose one group is moderate and CREE group two is light moderate.

Upper image- day 3
Lower images- day 21
Incision Wound Healing Activity of Cree

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Histopathology of Incision Wound Model

Histopathology of granulation tissue incision wound model is shown in Figure. A-Control, showing fibroblast and indistinguishable collagen fiber. B- Soframycin 25 mg/kg showing high level of fibrosis as well as found collagen fibers, C- Dose one conc. 200mg/kg normally increased fibrosis and collagenation, D- Dose two conc. 400 mg/kg showing highly moderate collage nation and fibrosis.

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DISCUSSION

In an animal model, the wound healing properties of *C. revoluta* ethanolic leaf extract were investigated. Wound healing is a complex process that occurs after injury to the skin and other soft tissues of the

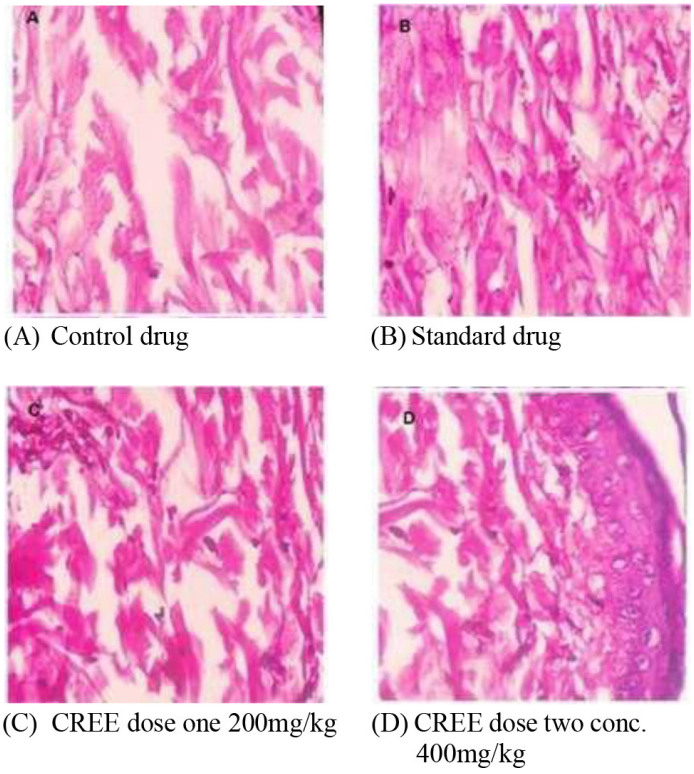

Figure 9: Histopathology of incision wound model (A) control Group (B) Standard group (C) CREE dose one group 200mg/kg and (D) CREE dose two group 400 mg /kg.

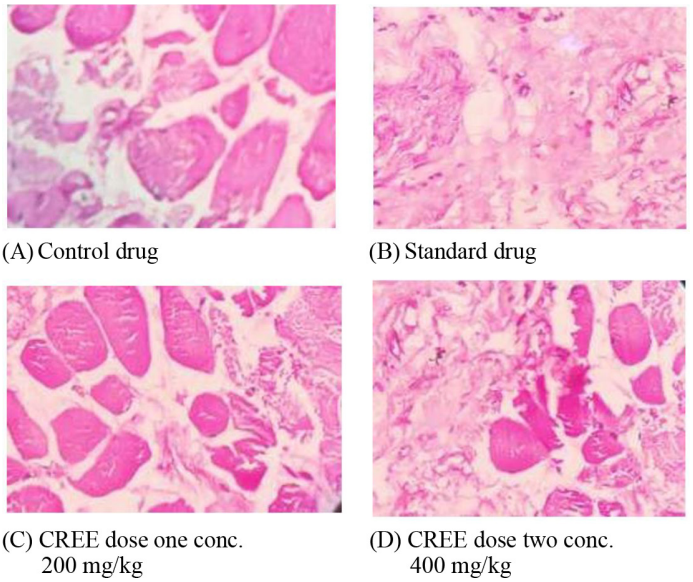

Figure 10: Histopathology of excision wound model (A) control Group (B) Standard group (C) CREE dose one group 200mg/kg and (D) CREE dose two group 400 mg /kg.

body. Wound healing is a dynamic process involving many biochemical processes aimed at restoring the injured cellular structure to its normal and original condition.[28] A typical wound healing cascade consists of three consecutive and overlapping phases: inflammation, proliferation, and remodeling.[29] In both the excision and incision wound models in rats, topical administration of prepared CREE (200mg/kg and 400mg/

kg) enhanced wound healing. The presence of alkaloids, flavonoids, terpenoids, glycosides, and tannins was observed during a preliminary qualitative phytochemical screening of the CREE. The CREE indicated a high level of phenolic and flavonoid content in the leaves of *Cycas revoluta*. Recent research has indicated that flavonoids, triterpenoids, and tannins play an important role in wound healing via a variety of mechanisms, including wound contraction, increased rate of epithelialization.[30,31] According to the present investigation, the wound healing effectiveness of CREE may be ascribed to its high phenolic and flavonoid content, which include astringent, anti-inflammatory, and antibacterial properties. The animals did not show any signs of restlessness or scratching/biting of the wound site when the *Cycas revoluta* extract was administered, indicating that it did not cause discomfort or pain. As previously said, the CREE has a high concentration of phenolic and flavonoids, which may have numerous mechanisms that aid in wound healing. Collagen is a crucial component that eventually plays a significant function in wound strength and tissue matrix integrity. It is a major extracellular protein in the granulation tissue of healing wounds.[32] The wound healing process is heavily reliant on the regulated synthesis and deposition of new collagens, as well as their maturation.[33] Wound contraction in CREE-treated showed improved venerability of collagen production, which might be attributed to the presence of flavonoid compounds,[34] whilst phenolic compounds may prevent subsequent wound infections owing to their antiviral and antibacterial properties.[35] In this work, we look at hydroxyproline levels as a biochemical measure of collagen turnover. Significantly increased ($p< 0.001$) hydroxyproline levels in the granulation tissue of extract (200mg/kg and 400mg/kg) treated rats indicate an elevated level of collagen content, resulting in rapid wound healing, and this venerable finding could be attributed to the presence of flavonoid.[36] The tensile strength of the treated wounds increased, which might be attributed to higher collagen levels and collagen fibers stability.[37] A histopathological examination of the extract treated rat wound tissues indicated that CREE enhanced wound healing.

CONCLUSION

In conclusion, the current study found that the CREE has phytoconstituents that support natural healing as well as anti-inflammatory action, and it may be utilised successfully as a wound healing agent. CREE effectively accelerates wound healing by increasing the rate of epithelialization, tensile strength, and collagen viability at the wound site. To fully comprehend the mechanism of *C. revoluta* extract's wound healing action, further studies are necessary. Finally, it was determined that topically administering *C. revoluta* leaf extract to rats was safe, with no evidence of drug-related toxicity.

ACKNOWLEDGEMENT

The authors are grateful to the institution for providing all of the necessary resources to make the experiment go well.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

Authors' Contributions

All authors have read and approved the final manuscript. AKS and SS designed the work. DRS drafted the work or substantively revised it. SKS contributed to the acquisition of the data. AM did the data analysis and interpretation AKT provided all the facilities and Technical support for pharmacologial work.

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History: Submission Date: 27-11-2021; Review Completed: 05-09-2022; Accepted Date: 12-11-2022.
Cite this article: Srivastava S, Sharma DR, Srivastava AK, Mukerjee A, Tripathi AK, Singh SK. Anti-inflammatory and Wound Healing Activity of Gymnospermous Plant *Cycas revoluta*. Indian J Nat Prod. 2022;36(1):44-51.