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SCREENING OF THE STEM BARK OF MORINGA OLIEFERA FOR IT"S ANTIMICROBIAL AND WOUND HEALING ACTIVITY

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ABSTRACT

Moringa oleifera is a pan-tropical species that is known by such regional names as benzolive, drumstick tree, kelor, marango, nébéday, saijhan, and sajna. Over the past two centuries, many reports have appeared in scientific journals describing the nutritional and medicinal properties of it. Its utility as a non-food product has also been widely explained it is used as lumber, charcoal, water clarification, lubricating oil. Different parts of the plant contains essential minerals and are a good source of protein, vitamin, amino acids and various phenolic compounds.. It has more than 40 natural anti-oxidants. The leaves, pods, seeds, gums, barks and flowers of Moringa are used to relieve mineral and vitamin deficiencies, support a healthy cardiovascular system, promote normal blood-glucose levels, neutralize free radicals {thereby reducing malignancy}, provide excellent support of the body's anti-flammatory mechanisms, enrich anaemic blood and support immune system. It also improves eyesight, mental alertness and bone strength. It has potential benefit in malnutrition, general weakness, lactating mothers, menopause, depression. There is a need to explore therapeutic, nutritional and benefit of this gift of nature reported to be one of the world's most useful tree. The present work comprises of research work wound healing potential and anti-microbial activity of stem bark of *Moringa oleifera*. Wound healing activity was performed by Incision wound model and Excision wound model. In incision wound healing model, the tensile strength of the 2% and 5% test group and the povidone-iodine ointment treated group were compared to each other. Tensile strength of 5% exact ointment showed significant results and 2% extract ointment showed lesser but significant increase in tensile strength compared to the control group. Thus both concentrations of the extract as well as the standard drug showed a

significant increase in tensile strength on the 14th day. In excision models the animals treated with the 5% w/w *Moringa oliefera* ointment shown to healed completely as compared to 2%w/w which was also at the complete healing stage as compared to control treated and the standard drug (povidone-iodine) treated group. The epithelization period of standard group and treated group was less in comparison with that of simple ointment base treated groups. The percentage of wound contraction was much more with the 5% w/w extract was similar to that of povidone-iodine treated group. 2%w/w treated group of animals showed significant wound contraction.

KEYWORDS: Moringa, traditional uses, benefits, nutritional value, wound healing, therapeutic use.

INTRODUCTION

Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae, that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. It is already an essential crop in India. All parts of the Moringa tree can be consumed. Moringa seed oil (yield 30-40% by weight), also known as Ben oil, is a sweet non-sticking, non-drying oil that can resists rancidity. It has been used in salads, also for fine machine lubrication, and in the manufacturing of perfume and hair care products. The leaves and buds of the plant are used as vegetable and can be rubbed on the temples for relieving headache while root and root bark are regarded as anti scorbutic and can be used externally as counterirritant.[1] The eye diseases are treated with the juice of the leaves with honey.[2] The plant is also known to possess high nutritional value and is used in a folklore medicine to treat various ailments related to pain and inflammation.[3] Dried seeds of *Moringa oleifera* are used in ophthalmic preparation, venereal affection anti-inflammatory, purgative and as tonic. The alcoholic extract of the leaves of *Moringa oleifera* are reported to have analgesic activity[4] and the aqueous extract of *Moringa oliefera* roots also shows antifertility profile.[5] The plant is reported to possess wide range of pharmacological effects that include antitumor[6], antispasmodic, diuretic[7], antiulcer[8], hypotensive[9], hypolipidemic[10], Hepatoprotective[11], antifungal[12] and antibacterial activities.[13]

Collection of Plant Part

The whole plant was gathered from the herbal garden of NIET college, Greater noida. The whole plant was collected.

Drying of The Parts of *Moringa Oleifera*

The plant was washed Roots along with other parts were discarded and the stem bark was sliced into small pieces and spread in thin layers in trays and dried the stem bark at room temperature for 3 days. Then the stem bark was dried in hot air oven at 50 °C for 3 days and at 40°C for the next 4 day. The dried parts were grind to coarse powder in the mixer- blender. This breaks the plant parts to smaller pieces which helps in easy penetration of extract in the cells. Then the powdered sample was kept in clean and closed glass containers till when the extraction was to be done. During grinding of sample, the grinder was clean to avoid contamination with any extract of previously ground material or other matters deposited on the grinder.

Incision Wound Healing Model

The experimental animals were groups into four containing six in each and treated as follows:

- Group I : Control
- Group II : povidone-iodine ointment (Standard)
- Group III : Received test ointment (2%w/w)
- Group IV : Received test ointment (5%w/w)

Animals in each group were anaesthetized and one para vertebral-long incision was made through the skin and cutaneous muscles at a distance of about 1.5 cm from the midline on each side of the depilated back of the rat. Full aseptic measures were taken. All the groups were treated in the same manner as that of excision wound model. After the incision, the parted skin was kept together and stitched with black silk at 0.5cm intervals; surgical thread (No. 000) and a curved needle (No. 11) were used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. The wound was left undressed. Sample extract along with simple ointment (control) and standard drug were topically applied daily for 14 days; when wounds were cured the sutures were removed on the 10th day and tensile strength was measured with a local made Tensiometer.

Excision Wound Model

The experimental animals were groups into four containing six in each and treated as follows:

- Group I : Control
- Group II : Received Povidone-iodine ointment (Standard)
- Group III :Received test ointment, (2% w/w)
- Group IV : Received test ointment, (5% w/w)

Rats were anaesthetized by open mask method with anaesthetic ether. The rats were depilated on the back and a predetermined area of 500 mm2 full thickness skin was excised from the dorsal inter scapular region. Rats wounds were left in open environment undressed for the monitoring of wound contraction and epithelisation time. The standard drug (Povidone-iodine), white petroleum jelly. *Moringa oleifera* methanolic extract ointment (2% and 5%) was applied everyday till the completion of wound. The changes in the wound area was monitored by tracing the wound margin on a graph paper. The measurement of wound area on graph paper. Wound contraction was expressed as percentage reduction of original wound size. (Mukherjee., 2010).

ANTI-MICROBIAL ACTIVITY

Anti Bacterial Activity

M.I.C Determination

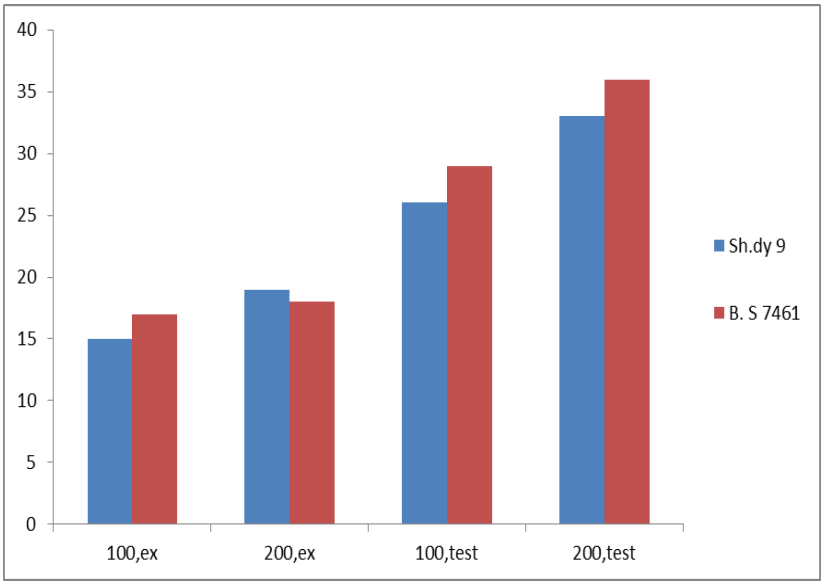
S.no	Mico-Organism	Dilution of methanolic stem bark extract in nutrient agar medium									
		0	5	10	25	50	100	200	400	800	1000
1	*E. coli ET/EC LT/SH*	+	+	+	+	+	+	+	+	+	+
2	*E. coli 306*	+	+	+	+	+	+	+	+	+	+
3	*E. coli 18/9*	+	+	+	+	+	+	+	+	+	+
4	*V.cholerae 765*	+	+	+	+	+	+	+	+	+	+
5	*V. cholerae 556*	+	+	+	+	+	+	+	+	+	+
6	*Shigelia boylii 22461*	+	+	+	+	+	+	+	+	+	+
7	*Sh.dysentera e 9*	+	+	+	-	-	-	-	-	-	-
8	*Salmonella aureus ML 267*	+	+	+	+	+	+	+	+	+	+
9	*Salmonella typhi*	+	+	+	+	+	+	+	+	+	+
10	*Bacillus subtilis 7461*	+	+	+	+	+	-	-	-	-	-

The analysis on the table shows the antibacterial potentiality of the stem bark of the plant. The methanolic extract was shown to inhibit strains of *Baccilus subtilis 7461* within 50 µg/ml- 1000µg/ml and strains of *Sh.dysenterae 9* were inhibited at 25µg/ ml-1000µg/ml. The strains of *Shigelia boylii, Salmonella aureus ML267, Salmonella typhi, V.cholerae765, V.cholerae 556 E. coli 306, E. coli ET/EC LT/SH, E. coli 18/9*were found to be completely resistant.

Determination of Zone of inhibition.

S.NO	Name of Bacteria	EXTRACT (µG/ML)		CIPROFLOXACIN (µG/ML)	
		TEST	(MM)	TEST	(MM)
		100	200	100	200
1	*Sh.dysenterae 9*	15	19	26	33
2	*Bacillus Subtilis 7461*	17	18	29	36

Determination of zone of inhibition in mm produced by extract of *Moringa oleifera* and its comparison with ciprofloxacin.



The disc diffusion (100 µg/ml. and 200 µg /ml) method was used to determine the zone of inhibition in comparison to ciprofloxacin as a standard against *Sh.dysenterae 9*, *B.subtilis 7461* were found to show good inhibitory results at 100 µg/ml, 200 µg/ml in (MIC) method. Further confirmatory tests using the disc diffusion method was performed on these bacteria which showed positive results in comparison with that of standard drug (ciprofloxacin). Result suggests that the methanolic extract of *moringa oleifera* exhibited significant antibacterial activity against all these bacteria.

ANTIFUNGAL ACTIVITY

M.I.C Determination

S.NO	Name Of Fungi	Dilution of Methanolic Extract of Leaves Parts (µG/ML) In Sabouraud's Dextrose Agar (Sda) Media									
		0	100	200	300	500	1000	1500	2000	3000	400
1	*Candida albicans ATCC-10231C7*	+	+	+	+	+	-	+	+	+	+
2	*Penicillium chrysogenum MTCC 2725*	+	+	+	+	+	-	-	-	-	-
3	*Candida albicans 5*	+	+	+	+	+	+	-	-	-	-
4	*Penicillium notatum ATCC 6259*	+	-	+	+	+	+	+	-	-	-
5	*Aspergillus niger MTCC 281*	+	+	+	+	+	+	+	+	-	-

0= control, ± Inhibited Growth, + Growth, - Absence of Growth

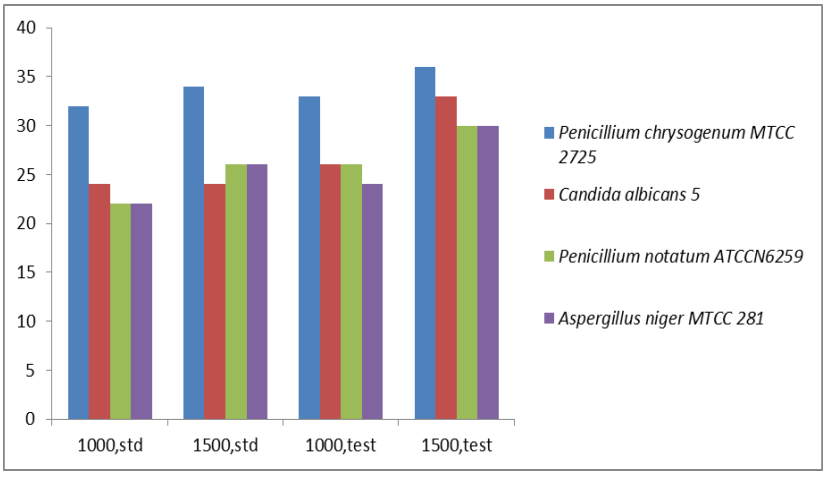
Determination of MIC of the methanolic extract of the stem bark of *Moringa oleifera* against different fungal strains:

The methanolic extract of the stem bark part of *Moringa oleifera* highly active against the most active against the most of strains which were used as the test micro-organisms. Candida albicans 5 1500 - 4000µg/ml and *Penicillium chrysogenum MTCC 2725* shows inhibiton at MIC of 1000- 4000 µg/ml. MIC for *Penicillum notatum ATCC 6259* species shows inhibition at MIC of 2000- 4000µg/ml.*Aspergillus niger MTCC 281*species shows inhibition at MIC of 3000-4000µg/ml.

Determination of Zone of Inhibition.

S.No	Name of Fungi	Griseofulvin (µG/ML) (STANDARD)		EXTRACT (µG/ML) TEST	
		1000	1500	1000	1500
1	*Penicillium chrysogenum MTCC 2725*	32	34	33	36
2	*Candida albicans 5*	24	24	26	33
3	*Penicillium notatum ATCCN6259*	22	26	26	30
4	*Aspergillus niger MTCC 281*	22	26	24	30

Determination of diameter of zone of inhibition in mm produced by the methanolic extract of stem bark of *Moringa oleifera* and its comparison with Griseofulvin against sensitive fungal strain:



The result of determination of zone of inhibition in comparison with the methanolic extract of stem bark of *Moringa oleifera* and comparison with standard antifungal agent Griseofulvin against the fungal strains is recorded in the above. Maximum zone of inhibition was obtained

with methanol extract against *Penicillium chrysogenum MTCC 2725* and *Candida albicans 5* against other tested fungi. Results suggest that the methanolic extract from the stem bark parts of *Moringa oleifera* exhibited significant antifungal activity against all tested fungi.

WOUND HEALING ACTIVITY

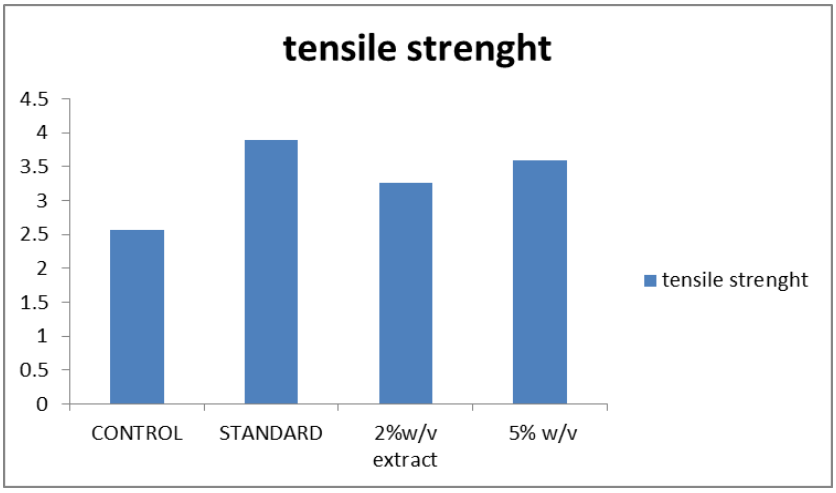
Incision Wound Healing Model

Better healing pattern and tensile strength was observed for the treated group on 10th day and was found to be significant than control group as shown in Table.

S.no	Group	Tensile strength (gm/mm2) (mean±sem)
1	Control	2.56±0.33
2	Standard	3.89±0.064
3	2%	3.26±0.17
4	5%	3.60±2.7

Evaluation of MOME on incision wound model in rats.

Each value represents the mean±SEM, n=6. comparison was done by ANOVA, followed by Dunnet s t-test. The tensile strength of the 5%w/w extract treated group and the Standard ointment treated group were compared with each other. The 5%w/w extract ointment treated group showed noteworthy increase in tensile strength as compared to the control group (2.56)gm/mm2. The tensile strength of 5%w/w treated group was (3.60) gm/mm2.both concentrations of the extract and the standard drug showed a considerable increase in tensile strength on the 10th day.



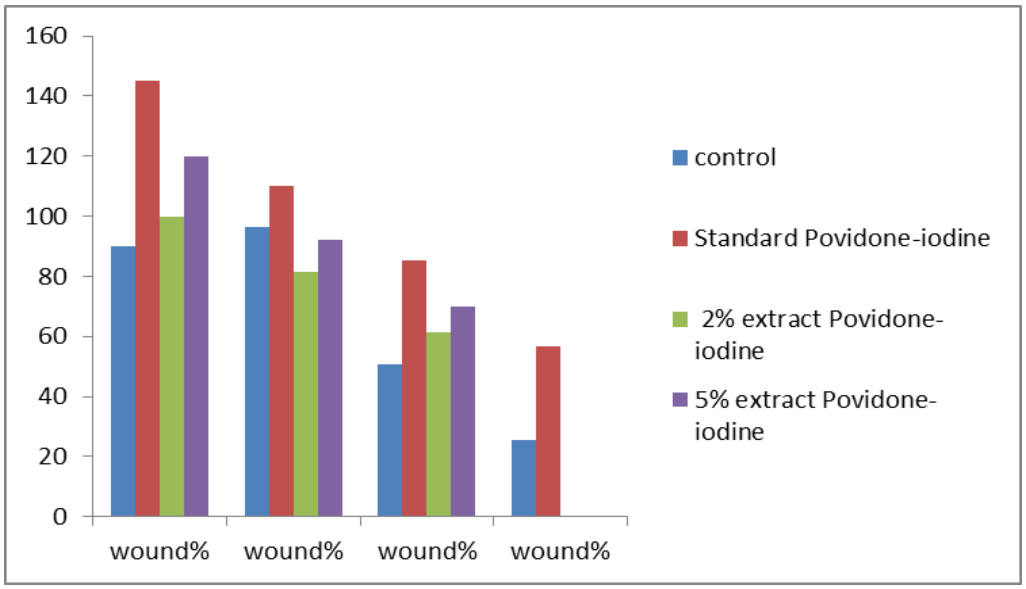
Excision Wound Healing Model

Topical application of the methanolic stem bark extract of *moringa oleifera* showed a significant wound healing activity as apparent by the lessening in the number of days

required for falling of the wound contraction as compared the standard. The results showed that the plant *Moringa oleifera* have an effective wound healing activity with the control group, there by explanatory use in the indigenous system of medicine.

Post wound week	Wound %			
	Control	Standard Povidone-iodine	2%	5%
1	112±3.0	145±1.04	100±2.5	120±5.00
2	96.5±1.5	110±7.0	81.5±6.32	92±2.54
3	50.57±5.0	85.4±2.5	61±5.68*	70.10±4.38**
4	25.6±0.52	56.48±1.04	-	-
Epithelisation days	30	20	25	22

Percentage of wound contraction



The ability of the ointment for the progression of the wound healing by, and the control groups (i.e. simple ointment treated groups). Changes in wound area was calculated by giving an suggestion of the rate of wound contraction. The areas of the wounds was measured by tracing the wounds on to a graph paper on the day of wounding and subsequently on 7th, 14th, 21th and 28th day post wounding. The number of days required for falling of the scar without any residual raw wound, is known as the period of epithelization.

In the excision wound method. It was experiential that the wound contracting capability of the extract sample in the form of ointment was considerably greater than that of the control. It was observed that time of epithelization of standard group and treated group was less as compared with that of group treated with simple ointment.

RESULTS AND DISCUSSION

The developed countries demand for more. Alternative treatments based on plants and they are becoming more famous and popular in India and other developed countries. *Moringa oleifera* stem have great potential to make possible and increase wound healing due to its anti-oxidant, anti-bacterial and other properties because of the various chemical metabolites present in the *Moringa oleifera*. *Moringa oleifera* is a capable compound when it comes to wound healing and care and could prove better results than obtainable drugs which are already present in the market. it may prove to be affordable and inexpensive drug with herbal origin which is presnt in the nature for free. Ointment formulation containing methanolic extracts of *Moringa oleifera* established was found to be significant in wound healing activity which was exemplify by rapid wound healing and contraction rates and reduced epithelization period. This study confirmed that methanolic extract of stem bark of *Moringa oleifera* was competent in promoting and accelerating wound closure process. The complex process of healing involves various factors like wound contraction and granuloma formation. wound contraction plays a noteworthy role in healing of excision wounds and granuloma formation helps in restored incision wounds.

Incision models are more relevant and are mostly use because of its resembles with most of the surgical wounds. The plant extract contains vitamin E, caratenoid, Fe, Zn and amino acids along with niazirin I and niazirin II and glycosides that helps in promotes wound healing process by the Application of the stem bark extract ointment which prevented the attack of the wound site by microorganisms, thus protecting the wound from being infected from the micro-organisms thereby enhancing speedy wound healing process. Even lower concentration of methanolic ointment used appeared to encourage wound healing process possibly along with the anti-microbial potential.

Histology of the injuries corroborate well with the measured wound contraction rate as this explains a attainable organic process result of the metabolites of Moringa extract used on animal tissue cells as discovered by the thickness and rate of the dermal covering, connective tissue resolution, healing proof by restoration of tissue design round the wound web site.

The potent antimicrobial activity of *Moringa oleifera* used in from this study further validates the previously reported antimicrobial property of.[14] The presence of flavonoids, saponins,

alkaloids and phlobatannins in the stem bark of *Moringa oleifera* could account for its antimicrobial activity.

The presence of flavonoid and saponins in this study correlates with the report of.[14] Flavonoids and tannins have been found to be effective against a wide range of microorganisms[15], while alkaloids are also known to impair enzymes that microorganisms use in energy production, thereby interfering with the integrity of their cell membrane and structural component synthesis.[16] This potential is due to the presence of biocompound benzyl-isothiocyanate which inhibit bacterial growth by disrupting the mechanisms of membrane and enzyme synthesis.[17] Also the antibacterial activity of *Moringa oliefera* extracts is also because of gallic acid and tannins[18], and saponins, tannins, isothiocyanates and phenolic compounds, such as alkaloids and flavonoids, which have inhibitory activity[19] which present in it. Many studies have resulted the antifungal activity of *Moringa oleifera* seed extracts against *Alternaria sp., Colletotrichum sp., Candida albicans and Fusarium sp, C. albicans and Aspergillus flavus*. The potent antimicrobial activity of *Moringa oleifera* used in from this study further validates the previously reported antimicrobial property. *dermatophytes, rubrum, Trichophyton, Trichophyton, mentagrophytes, Epidermophyton floccosum and Microsporum canis* which were isolated from clinical samples, as well as Candida species, such as *C. famata, C. tropicalis and C. ciferri* isolated from prawn farming.[20] M. canis isolated from cases of feline dermatophytosis, as well as C. albicans from the oral microbiota of dogs were also susceptible to flower and seed extracts. The antifungal activity of this essential oil was because of polyphenols, hydrocarbons, hexacosane, pentacosane, heptacosane, phytol and thymol.[21] Moreover, flavonoids and the compounds pyterigospermin and isothiocyanates obtained from seeds and leaves of Moringa also shows antimicrobial activity.[22]

The analysis on the shows the antibacterial potentiality of the stem bark of the plant. The methanolic extract was shown to inhibit strains of *Baccilus subtilis 746* and strains of *Sh.dysenterae 9* were inhibited The strains of *Shigelia boylii, Salmonella aureus ML267, Salmonella typhi, V.cholerae765, V.cholerae 556 E. coli 306, E. coli ET/EC LT/SH, E. coli 18/9*were found to be completely resistant. The study verified Moringa oleifera stem bark extract against *Sh.dysenterae 9, B.subtilis7461* found to show good inhibitory results as it exhibited significant antibacterial activity against all these bacteria. *Moringa olifera* provides good healing effect in different types of wound also it could be promoting natural anti-

bacterial agents with potential applications in pharmaceutical industry. So it can be formulated into suitable formulation required. Correct clinical management is required to provide rapid healing and and to reduce potential complications. *Moringa oleifera* having rich amount chemical metabolites like flavonoids, tannins and anthocyanin. These constituents are potential to assist the anti-oxidant, anti-inflammatory and anti-bacterial properties so it more helpful in therapeutic practices. This research may be used to widen new wound healing formulations for the use human being and to discover new herbal remedies for modern drug delivery. The tree as a native to India can become a great source of income for the nation if this potential for highly nutritional food is exploited by the industries and researchers by undertaking further research to corroborate earlier studies.

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