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Original Article

Oryza Sativa L. Indica Ointment Effect on Histopathological Skin and Collagen Features in Ultraviolet B-Exposed Rattus novergicus

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ABSTRACT

Outdoorsy folks get UV radiation. Acute UVB decreases hyaluronic acid. Antioxidants can prevent photoaging by delaying, stopping, or eliminating oxidation-induced molecular damage. Black rice extract ointment (Oryza sativa l. indica) was tested on the histological features of skin and collagen in Wistar rats (Rattus norvegicus) subjected to ultraviolet B radiation. This study is lab-based. The research samples were Wistra strain white mice (Rattus norvegicus). Reaserch with treatment groups is Precondition variables (UVB rays), independent factors (10%, 20%, and 30% black rice extract ointment), and fixed variables (dermis collagen and histological picture of mice exposed to UVB rays) were the research variables. The research procedures included the accreditation of test animals, black rice extract, ointment, phytochemical screening, treatment, histopathological skin tissue preparations, and histopathological observation. All the data was examined with SPSS. The investigation revealed that 30% black rice extract ointment had the highest collagen density (mean and SD = 55,959 ± 2.5). All groups K-, K+, P1, P2, and P3 had significant data normality tests of 0.200 > 0.05. The homogeneity p-value is 0.06 > 0.05, and the sig (2-tailed) value is 0.000 < 0.05 in the t-test. The alkaloids, flavonoids, tannins, and steroids in black rice extract increase glutathione peroxidase levels in mice, which helps collagen density when exposed to UVB light at 125 mg/kg BW.

Keywords: *Collagen, Black Rice Ointment, UVB Radiation, Histopathological Skin*

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INTRODUCTION

Aging is an unquestionably natural phenomenon characterized by biological processes that result in the steady build-up of harm, ultimately culminating in illness and eventual death [1,2]. With advancing age, the skin changes, such as increased dryness, paleness, translucency, and fragility [3].

Human skin protects the body [4,5]. The epidermis and dermis are thin and flattened with age, leaving the skin less firm and smooth. Old age generates fine lines, pigment spots, sagging, and wrinkles. Due to intrinsic and extrinsic aging causes, the complex physiology, biochemistry, and structural integrity of the dermis in aged skin changes rapidly [6].

Time and genetics cause intrinsic or chronological skin aging. Extrinsic skin aging is mainly induced by UV radiation or photoaging [7]. Both aging processes are linked to sun-exposed skin and cause wrinkles, sagging, and skin brittleness due to dermal matrix changes [8].

Solar ultraviolet (UV) activates MMPs, which degrade collagen, fibronectin, and elastin, causing skin aging [9]. After acute UV exposure, the activation of significant cell transcription factors enhanced MMPs 1, 2, 3, and 9 synthesis and other cellular processes linked with decreased type I procollagen

synthesis [10]. Mutations and skin cancer result from UV radiation-induced DNA damage [11,12]. UV radiation stresses epidermal tissue and enters the dermis. Basal cell carcinoma, malignant melanoma, and squamous cell carcinoma are linked to cumulative UV radiation exposure [13].

UVB-induced soluble mediators from keratinocytes, such as specific cytokines and matrix Metalloproteinases (MMPs), penetrate lower into the dermis and impact the extracellular matrix (ECM) [14,15,16]. Aging promotes skin moisture and epidermal Hyaluronic Acid (HA) loss, which stores water [15].

Photoaging causes uneven epidermal thickness and shape, unlike intrinsic aging [15, 16,17]. Photodamaged skin is thicker than organically aged skin. Melanogenesis is increased and neutralizes UV-induced free radicals, which may protect against photodamage [8].

As humans age, natural (intrinsic) and environmental (extrinsic) factors reduce the production of these essential components, causing skin deterioration, wrinkles, and sagging [18]. The loss of collagen I in elderly skin makes collagen seem disordered and increases the collagen III-collagen I ratio [3].

In healthy skin, the extracellular matrix mainly consists of glycoproteins such as collagen, elastin, fibronectin, laminin, and proteoglycans/glycosaminoglycans [19]. The dermis' primary components—collagen, elastin, and hyaluronic acid—make skin soft and elastic. Collagen strengthens and firms skin [20, 21, 22]. Elastin keeps skin stretchy. Hyaluronic acid plumps the skin matrix with water, making it fuller, firmer, and more youthful [20].

Outdoor activities can expose people to UV radiation, which can accelerate aging, especially during peak sun hours from 10 am to 4 pm, and wear UV-blocking garments, hats, sunglasses, and sunscreen [3].

Antioxidants can also prevent photoaging by slowing, stopping, or eliminating molecular damage from oxidation processes. Endogenous enzymatic antioxidants like Glutathione peroxidase, SOD, catalase, and low molecular weight non-enzymatic antioxidants, including GSH, uric acid, and ubiquinol, protect the skin. Extreme UV exposure can deplete the skin's antioxidant reserves. Hence, external antioxidants are needed, such as antioxidant-rich natural products. Antioxidants are found in black rice (*Oryza sativa l. indica*) [23, 24, 25]

Black rice (*Oryza sativa l. indica*) is nutritious and antioxidant. Black rice contains antioxidants such as phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid [26].

Beneficial black rice is colored. Essential amino acids, functional lipids, dietary fiber, vitamins, minerals, anthocyanins, phenolic compounds, tocopherols, tocotrienols, phytosterols, and phytic acid are found in this rice [27,28]. This plant's substance can inhibit photoaging-causing free radicals.

Several previous studies have discussed the effects of black rice (*Oryza Sativa L. Indica)*, such as research on the impact of applying black rice extract to lipid-based food products, where the research results show the quality and safety of applying natural antioxidants to food products [29]. Then, research was conducted into black rice as an active chemical that is an antioxidant and protects against UV radiation [30], as well as research on black rice bran, which can be used as a skin-lightening agent [31]. In this study, black rice extract will look at its effect on collagen and the histopathological features of the skin of obese mice exposed to UVB Rays.

METHOD

This is true experimental research using Post Test Only Control Group Design, which only observes the control and treatment groups after an action [32]. Inclusion criteria were male Wistar strain (*Rattus norvegicus*) rats weighing 200-300 grams, 2-3 months old, healthy physical condition, no anatomical abnormalities, and never used as research samples before. The exclusion criteria were white mice that died or were disabled during the experiment [33]. Samples were calculated using the Federer formula $(n-1) \times (t-1) \geq 15$ [34]. Federer's sample calculations showed that each group needed 5 test animals. Each experimental group had 6 Wistar mice, totaling 30 mice. Since rats (Rattus norvegicus) share so many physiological and behavioral traits with humans and are among the most popular research animals in the biomedical sciences, scientists have found them to be an ideal model for human subjects. Mice of the white variety also do well when housed in a controlled laboratory setting [35].

The precondition variable, Ultraviolet-

B light; the independent variable, Black Rice (*Oryza sativa l. indica*) Extract Ointment at 10%, 20%, and 30% concentrations; and the fixed variables, dermis collagen and histopathological skin tissue, were the variables in this study.

The research includes water baths, rotary evaporators, filters, rat drums, drinking containers, shavers, digital scales, surgical instruments, microscopes, rulers, cameras, and maceration tools. We will need the following items for in vitro testing: 10 ml, 25 ml, and 100 ml measuring flasks; test tubes; test tube racks; BioHit 1000 µL micropipettes; measuring pipettes; spatulas; vials; incubators; pH meters; cuvettes; centrifuges; centrifuge tubes; UV-spectrophotometers Vis; beaker glass; and an ultraviolet lamp B PL-S9W/01/2P. Brand Philips. Trichloroacetic Acid (TCA), ethanol propionate (Brataco), Aquades, Sirius red, and black rice extract simplicia (*Oryza sativa l. indica*) are some of the compounds utilized in the research.

The research procedure was to acclimate test animals for seven days at the Animal House, Faculty of Mathematics and Natural Sciences, University of North Sumatra. Then, the extract is made by preparing black rice (Oryza sativa l. indica) from Medan farmers, separating bad rice, washing, and drying it in a 50^{o}C oven. The simplicial powder is blended and sieved with a 40-mesh sieve. Macerate and filter 96%: HCl (9:1), 1 part bran: 10 parts solvent to make black rice extract ointment at 10%, 20%, and 30%. The filtrate was evaporated in a rotary evaporator at 40°C, 50 mBar vacuum pressure, and 100 rpm to provide a crude extract for phytochemical analysis. Mix the extract and base ointment until homogenous.

Then, make two ointments: one without extract and one with black rice extract (*Oryza sativa l. indica*). The basic or control ointment contains 170 gr soft paraffin, 10 gr hard paraffin, 10 gr cetostrearyl alcohol, and 10 gr wool fat—an ointment containing 10%, 20%, and 30% black rice extract. Then, test the secondary metabolites for the content of tannins, flavonoids, alkaloids, steroids/terpenoids, and saponins in black rice extract ointment (*Oryza sativa l. indica*).

Add 1 gram of extract to a test tube, add 10 mL of hot water, boil for 5 minutes, then add 3-4 drops of FeCl3 to the filtrate for the Tannin Content Test. It is positive for catechol tannins if it is blue-green (green-black) or blue-black. pirogalo tannin. In the Flavonoid Content Test, 1 gram of sample extract was added to a test tube, concentrated HCl was added, and the tube was heated for 15 minutes in a water bath Red or yellow indicates flavonoids (flavone, chalcone, aurone). The Alkoloid Content Test involved dripping 5 mL of 2 N HCl over 2 grams of sample extract, heating, cooling, and dividing into 3 1 mL test tubes. Reagents are added to each tube. If Mayer's reagent precipitates white or yellow, alkaloids are present. Wagner's reagent detects alkaloids if a brown precipitate appears. The Dragendrof reagent contains alkaloids and gives an orange precipitate. In the Steroid/Terpenoid Content Test, 2 grams of sample extract were shaken in a test tube with 2 mL of ethyl acetate. The ethyl acetate layer was dropped onto a drop plate to dry. After drying, 2 drops of acetic acid and 1 drop of concentrated sulfuric acid were added. Terpenoids are present if they become red or yellow. Steroids are present if they turn green. In the Saponin Content Test, 1 gram of sample extract is added to a test tube, 10 ml of hot water is added, cooled, and shaken violently for 10 seconds. Saponin is present if the foam is 1-10 cm high in 10 minutes and does not dissolve after adding 1 drop of 2 N HCl.

Male Wistar rats acclimated and fed pellet food were randomly divided into five groups. All 30 mice were exposed to ultraviolet-B light daily for 28 days in 5 groups. The Negative Control Group (K-1) exposed mice only to ultraviolet-B light, the Positive Control Group (K-2) treated mice with basic ointment, the Treatment Group-3 (K-3) treated mice with 10% black rice extract ointment, the Treatment Group-4 (K-4) treated mice with 20%, and the Treatment Group-5 (K-5) treated mice with 30% black rice extract (*Oryza sativa l. indica*).

Then, proceed with making Histopathological Skin Tissue Preparations; Melanin appears as tiny black dots when viewed via a microscope. After taking pictures of the parts that came up positive, we ran the images through Image J for analysis. The Histopathological Observation Process using a scoring system viewed using a light microscope with 400x magnification.

Histopathological tissue metrics will include skin collagen density and melanin pigment. After all treatments, the researchers took a biopsy of the mice's back skin (2 cm x 2 cm x 2 mm). They stained it with Picro Sirius

Red to measure dermis collagen density and Fontana-Masson to measure melanin.

The scoring system used is based on collagen density from the study as follows: 0 = normal, 1 = mild increase, 2 = moderate increase, and 3 = significant increase [36]. The amount of collagen is calculated as the percentage of red collagen area pixels compared to the entire tissue's pixel area [37] . Here are three categories for melanin pigment that will be counted: little (<40 melanin pigments), moderate (40-80 melanin pigments), and a lot (>80 melanin pigments). The melanin pigment visible under a microscope with interpretation will be used [38].

Data in this study was normalized using the Kolmogorov-Smirnov test. P > 0.05 indicates normal distribution. After normalizing the data, use Levene's test for homogeneity. P > 0.05 indicates homogeneity [39]. The SPSS analysis test tool uses the t-test to compare groups after normality and homogeneity testing.

RESULTS

The test animals mainly were male Wistar rats (*Rattus norvegicus*) of the Wistar strain. They were averaging 200-300g and 2-3 months old. Black rice extract ointment (*Oryza sativa l. indic*a) is applied after the disinfected rat's back is shaved. Each rat was shaved with a 4x4 cm hair shaver. Treat each group. Each ointment concentration was administered at 0.1 ml/cm2 to the backs of shaved mice subjected to ultraviolet B light. In ultraviolet B light, the ointment was applied twice daily for four weeks, 20 minutes after exposure. Violet B was applied four hours later at 09.40 AM, 10.00 AM, and 2.00 PM. On radiation-free days, ointment is used.

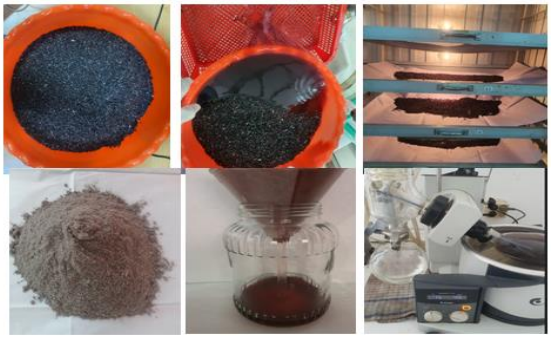

Figure 1. Process for Making Black Rice Extract

From selecting the best black rice to manufacturing black rice extract, Figure 1 demonstrates the process. Put black rice flour in a glass bottle after grinding to 60 mesh. The extraction was done in a light-tight glass bottle at room temperature (± 27 °C) for 24 hours. After filtering the crude extract, a rotary vacuum evaporator at 60 °C evaporated the filtrate. The final thick extract (± 10% of total solvent) was kept at 4 °C for further analysis.

Making Black Rice Extract Ointment with mixtures of extracts of 10%, 20%, and 30% are prepared by levigationally mixing accurately weighed extracts into the base ointment to make a fine paste with 2 or 3 times the base weight, gradually adding more base ointment until it forms a homogeneous ointment, and finally packaging the mixture. In the organoleptic test (blank, F1, F2, F3), the black rice ointment extract was odorless, white, light brown, brown, homogeneous, solid, and pH 5.37 - 6.33, suitable for human skin. The ointment spreadability test showed a 5.0 – 5.9 cm diameter in 0 – 125 gr packages. Thus, the ointment is safe for experimentation.

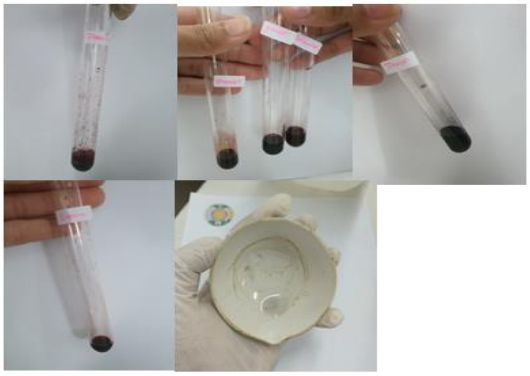

Figure 2: Phytochemical test results

Phytochemical studies have shown that the alkaloids, flavonoids, tannins, and steroids found in black rice extract (*Oryza sativa L. indica*) increase glutathione peroxidase levels in mice, suggesting that this extract may have antioxidant properties.

Based on the information in Table 1, we can observe that the control group (K-) of mice that did not receive any treatment other than exposure to UVB light for 28 days had an average percentage of collagen density of 36.37 ± 0.8. In the treatment group (K+), mice were exposed to UVB light and given basic ointment without black rice extract, resulting in an average result of 42.25 ± 1.3. In treatment group 1 (P1), mice were exposed to UVB light and

given a 10% concentration of black rice extract ointment daily for 28 days. In this group, the average percentage of collagen density was 45.59 ± 2.8. Lastly, in treatment group 2 (P2), mice were exposed to UVB light and given a 10% black rice extract ointment concentration. For treatment group 3 (P3), the average result with a standard deviation was 50.30 ± 1.2. Mice in this group were subjected to UVB and daily applications of a 30% concentration of black rice extract ointment for one month. A standard deviation accompanied the 55.96 ± 2.5 average result.

Our results suggest that the P3 group received a 30% concentration of black rice extract ointment as treatment and had the highest average percentage area of collagen density. The negative control group has the lowest average collagen density percentage area if that's true. Specifically, the group that underwent treatment with UVB rays but did not get any black rice extract ointment.

Table 1. Collagen Density Test Results in Mice Skin Tissue

Repetition	Treatment Group				
	K-	K+	P1	P2	P3
1	37.26	42.61	48.93	51.06	58.03
2	35.38	39.87	42.42	48.99	53.96
3	37.37	43.17	46.27	50.47	57.31
4	36.33	43.79	42.94	48.93	52.36
5	35.56	41.68	44.26	50.22	55.33
6	36.77	42.33	48.72	52.14	58.76
Score	0	1	2	3	3
Mean	36.37	42.25	45.59	50.30	55.96
SD	0.8	1.3	2.8	1.2	2.5

Note Score: 0 = normal, 1 = mild increase, 2 = moderate increase, and 3 = significant increase.

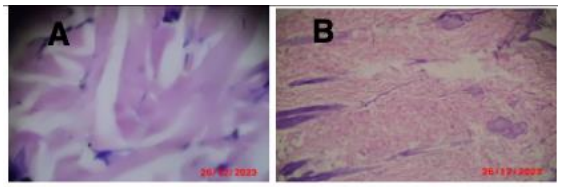

Figure 3. Histopathological Features of Collagen (A) and Melanin (B) in the Negative Control Group (K -).

With a collagen density (A) of 0, the negative control group (K-) exhibited a thick, typical look and a purplish-blue hue (Figure 3). Because these mice were solely exposed to UV-B radiation for 28 days and received no therapy, their collagen density is higher, and their fibers are not disseminated. The mouse epidermis also found many damaged skin melanin pigment (B) patches. Radiation intensity influences melanin pigment treatment.

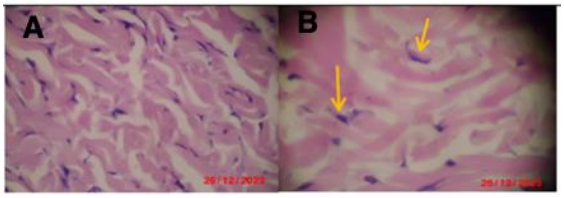

Figure 4. Histopathological Features of Collagen (A) and Melanin (B) in the Positive Control Group (K +).

Histology images of collagen density (A) in the positive control group (K+) indicated a little elevation of 1 point, a thick, purplish-blue appearance, and fibers started to scatter in the same direction (Figure 4). UVB rays were utilized on this subset of ointment without black rice extract. Granules formed in the mice's epidermis, indicating that the damaged melanin pigment (B) was working. The results show that base ointment melanin pigment production depends on exposure.

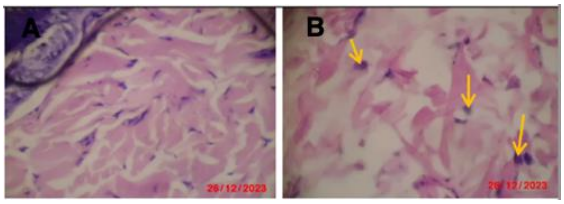

Figure 5. Histopathological Features of Collagen (A) and Melanin (B) in the Treatment Group 1 (P1).

In the initial treatment group 1 (P1), collagen density (A) had a score of 2 considerable increases, a dense and purplish-blue appearance, and distributed fibers (Figure 5). This group of mice received 10% black rice extract ointment and ultraviolet B light for 28 days. In mice, damaged skin melanin pigment (B) in the epidermis fades and has fewer granules—exposure to 10% black rice extract ointment daily for 28 days impacts melanin formation.

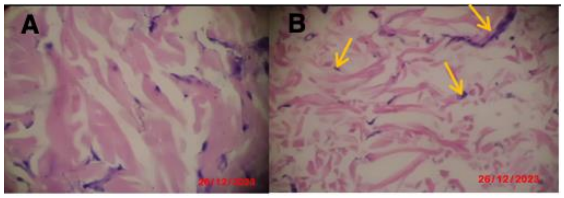

Figure 6. Histopathological Features of Collagen (A) and Melanin (B) in the Treatment Group 2 (P2).

On histopathological imaging, the second treatment group of mice (P2) had a statistically significant increase in collagen density (A) of 3 compared to the light-exposed control group (Figure 6). The image was predominantly purplish blue, and collagen density was high. Ointments with 20% UVB and black rice extract were applied daily for 28 days. Melanin pigment was found in wounded skin's smaller, fewer-granule epidermis. Daily application of 20% black rice extract ointment for 28 days shows that exposure influences melanin pigment production.

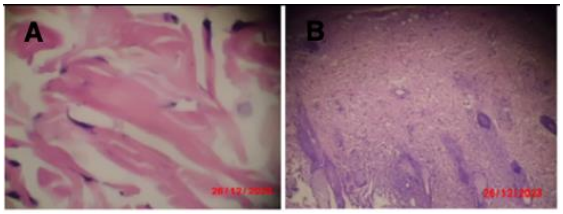

Figure 7. Histopathological Features of Collagen (A) and Melanin (B) in the Treatment Group 3 (P3).

In Figure 7, treatment group 3 (P3)'s histological collagen density (A) image resembles treatment 2, with a score of 3, suggesting a significant rise. Purple-blue picture dominates results. These mice were exposed to UVB rays and treated with 30% black rice extract ointment daily for 28 days. Microgranules of damaged skin melanin pigment (B) were found in the mouse epidermis. Applying 30% black rice extract ointment daily for 28 days shows that exposure influences melanin formation.

Table 2. Normality Test Result

Groups	Kolmogorov-Smirnov[a]		
	Statistic	df	Sig.
Control -	.184	6	.200*
Control +	.191	6	.200*
P1	.198	6	.200*
P2	.189	6	.200*
P3	.205	6	.200*

*. This is a lower bound of the true significance.
a. Lilliefors Significance Correction

The results demonstrate that all groups K -, K+, P1, P2, and P3 exhibit a significant value of 0.200, according to the image in Table 2 that has been normality checked using SPSS. If the p-value in the Kolmogorov-Smirnov Test is more significant than the standard margin of 0.05, then the data is considered regularly distributed.

Table 3. Homogeneity Test Results

Levene static	**df1**	**df2**	**Sig**
4.665	4	25	.006

The significant statistics in Table 3 indicate a p-value of 0.06. There is a statistically significant difference in the mean percentage of collagen density between the four groups, as Ho is rejected at the fundamental level = 0.05.

Table 4. T-test results

	One-Sample Test					
	t	**df**	**Sig. (2-tailed)**	**Mean Difference**	**95% Confidence Interval of the Difference**	
					Lower	**Upper**
Result	35.98	29	.000	46.11	43.4911	48.734

Table 4, the t-test results show that each group has an average t-value of 46.11. Since the sig (2-tailed) value is 0.000 < 0.05, it may be inferred that there is a significant difference between the pairs of groups.

DISCUSSION

Due to its alkaloids, flavonoids, tannins, and steroids, black rice (*Oryza sativa L. indica*) extract increases glutathione peroxidase in mice, according to phytochemical research. Antioxidant glutathione fights free radicals, slows aging, and boosts the immune system. According to Haryanto, black rice (*Oryza sativa l. indica*) is nutritious and antioxidant-rich [40].

Ointments of black rice extract are checked before testing samples. The organoleptic tests (Blanko, F1, F2, F3) showed that the black rice ointment extract was odorless, white, light brown, brown, homogeneous, solid, and pH 5.37–6, 33, suitable for human skin. The ointment spreadability test showed a 5.0–5.9 cm diameter

in 0–125 gr packages. Thus, the ointment is safe for experimentation.

CONCLUSION

Based on the research findings, it can be concluded that using 125g of black rice (*Oryza sativa l. indica*) extract ointment daily for 28 days at a 30% concentration yields average results with a standard deviation of 55,959 ± 2.5. This ointment is effective in healing skin wounds exposed to UVB light due to its superior melanin pigment synthesis and high collagen density. This is because antioxidants such as steroids, alkaloids, flavonoids, and tannins found in black rice extract help the body fight free radicals, delay aging, and keep the immune system strong.

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CONFLICTS OF INTEREST

All writers have stated no conflicts of interest in this publication.

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