Original Article

The Potential Protective Effect of Curcumin Nanoparticles on Radiated Tongue of Male Albino Rats (Histological and Immunohistochemical Study)

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ABSTRACT

Introduction: Despite radiotherapy being the most commonly used treatment for head and neck malignancies, most patients experience oral problems triggered by DNA damage and subsequent reactive oxygen species production (ROS). Curcumin, an antioxidant found in plant extracts, has recently received the focus of attention for its potential effectiveness against oxidative stress-related disorders, mainly when used in nanoparticles. The tongue is one of the vital organs that could be affected by radiation side effects.

Aim of Work: This study aims to evaluate the cytotoxic influences of radiation on the tongue mucosal tissues and assess the possible protective impact of curcumin nanoparticles (Cur NPs) on these irradiated tissues.

Materials and Methods: Randomly dividing thirty-six male albino rats into four groups, group I (the negative control) had no exposure to radiation nor treatment, group II (the positive control) received Cur NPs without radiation, group III (irradiated), group IV (irradiated+Cur NPs). The heads of rats in groups III and IV were exposed to 15 Gy of radiation. Rats in group IV received Cur NPs orally 48 hours before radiation and daily (100 mg/kg) for a week. At the experiment's end, tongue tissues were dissected and processed for both histological and immunohistochemical analysis.

Results: Histological examination of group III showed destructive changes in the tongue tissues, but group IV demonstrated greater re-epithelization with nearly typical histological features. Immunohistochemical results using anti-PCNA proved that Cur NPs displayed an improved cell proliferation rate, revealing a better healing effect in group IV compared with groupIII.

Conclusions. Radiotherapy produced histopathological changes and damage in the mucosal tissues of the tongue. However, the administration of Cur NPs before and after radiation has been proven to reduce these damaging effects through their potent antioxidant, anti-inflammatory, and immunomodulatory properties.

Received: 08 February 2023, **Accepted:** 13 April 2023

Key Words: Antioxidant, curcumin nanoparticles, radiation, reactive oxygen species, tongue.

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ISSN: 1110-0559, Vol. 47, No. 2

INTRODUCTION

Radiotherapy is the most prevalent head and neck cancer treatment; it employs ionizing radiation to destroy malignant cells and stop their growth. Although radiotherapy results in a substantial rise in cure rates for many malignancies, the healthy normal tissues exposed within the radiation field bring about several undesirable reactions[1]. The oral cavity is a common site for adverse impacts of radiotherapy. So, most patients have oral complications such as oral mucositis (O.M.), candidiasis, progressive loss of periodontal attachment, and jaw osteoradionecrosis[2-4]. O.M. is an inflammatory reaction of the mucous membrane during radiotherapy that occurs due to the impacts of radiation on the basal cell layer, composed of rapidly dividing radiosensitive cells[3,5]. Any mucositis during a cancer treatment period raises the risk of infections and bleeding, increasing the duration and cost of hospitalization[6]. Several studies reported that the pathogenesis of mucositis is due to the breakdown of DNA strands as the result of the production of reactive oxygen species (ROS) or the activation of transcription or enzymatic factors in mucosal cellular elements[7]. Antioxidants are substances that improve the immune system's function and reduce or eliminate the effects of ROS in the human body by preventing their creation or lowering their impact if they form[8]. Curcumin, which comes from the Curcuma longa plant, is one of these antioxidants and is mostly water-insoluble. Despite being an impeccably safe medication, curcumin's poor bioavailability, high rate of metabolism, rapid clearance, and reduced absorption in the gastrointestinal tract prevented it from achieving the best therapeutic results in previous clinical investigations[9,10]. Because of these drawbacks, some researchers have begun using curcumin nanoparticles to target the delivery of the drug, enhance its solubility, increase its bioavailability, and give curcumin higher permeability and stronger resistance to metabolic processes[11,12]. The tongue is one of the normal tissues that can be subjected to the secondary influence

 DOI: 10.21608/ejh.2023.191587.1852

of ionizing radiation during head and neck malignant treatment. Because of the importance of tongue tissues in speaking, mastication, taste, and exploring the effect of nanotechnology on the compounds from natural origin.

So, the current research attempts to evaluate the possible protective impact of Cur NPs on the irradiated tongue tissues specifically the filiform papillae and related lamina propria.

MATERIAL AND METHODS

Study protocol

The Ethics Committee approved the protocol for the animal experiments underlying this study at the faculty of Dentistry, Ain Shams University, Egypt. With ethical approval number FDASU-Rec IR092214, the experiment was performed in the animal house of Ain Shams University's medical research center. In this study, thirty-six male Albino rats weighing from 200 to 250 grams were used. To rule out any intercurrent infection and allow the animals to adjust to their new cages, they were kept under observation for roughly a week before the beginning of the experiment. The rats were kept in an environment with regulated temperature and lighting conditions. They were housed in separate cages and provided a standard diet of fresh vegetables, dried bread, and ad libitum tap water.

Animal grouping and dose administration

The sample size was calculated using G*Power statistical power analysis program (version 3.1.9.7)[13]. The total sample size was 36 (n=36).

Rats were divided randomly into 4 equal groups (n = 9 per group):

1. **Group I:** passive control group: rats received no radiation or treatment.
2. **Group II:** positive control group (Cur NPs treated group): rats received Cur NPs only without radiation.
3. **Group III** (irradiated group): rats were exposed to the radiation dose of 15 Gy.
4. **Group IV** (irradiated and treated with Cur NPs): rats were exposed to the radiation dose of 15 Gy and received Cur NPs.

Groups II and IV: rats received daily Cur NPs in powder form diluted in distilled water (1:6). The solution was administered via oral gavage (100 mg/kg) forty-eight hours prior to radiation, then once daily for a week[14,15].

Radiation Exposure

The radiation process was conducted at the National Centre for Radiation Research and Technology of the Egyptian Atomic Energy Authority in Cairo. Before radiation, the rats in groups III and IV were weighed and anesthetized intraperitoneally with xylazine (20 mg/kg) and ketamine (60 mg/kg)[16]. Only the heads of the rats were subjected to radiation, while the rest of their bodies were protected by lead cylinders. The radiation was done with a cobalt 60 gamma radiation source and was delivered to the rats' heads in one exposure at a source surface distance (SSD) of 70 cm[17].

Material

Cur NPs utilized in this study were purchased from Nanotech Egypt Chemical Company. The materials were characterized by using high resolution transmission electron microscope (HRTEM): JEM-2100 microscope, Faculty of Science, Ain Shams University. TEM was used to examine the size and shape of the prepared samples.

Sample preparation for histological examination

At the study's end (one week after irradiation), rats were sacrificed by an overdose of anesthesia (thiopental sodium, 80 mg/kg) via intraperitoneal injection[18]; the tongue was dissected out, washed in saline solution, and fixed in 10% buffered formalin.

a- Routine (H&E) staining: The samples were prepared for standard histological analysis according to Bancroft and Layton [19].

b- Proliferating cell nuclear antigen (PCNA) immunohistochemical staining: For the immunohistochemical study, the spreading of anti-PCNA receptor subunits in tongue tissues was defined in deparaffinized sections using an Avidin-Biotin-Peroxidase (Elite-ABC; Vector Laboratories, CA, USA) and anti-PCNA monoclonal antibody (dilution1:100; DAKO Japan Co, Tokyo, Japan). Sections were deparaffinized and washed in ethanol before being stained with PCNA. The slides were incubated in hydrochloric acid for 30 minutes at room temperature and then washed in Phosphate buffer saline (PBS). The slides were pre-incubated in 5% blocking serum for 30 minutes, then incubated in primary antibody diluted in PBS with 1% bovine serum albumin overnight at 4°C. After incubating those sections for 20 minutes at 42°C with a biotinylated horse anti-mouse IgG diluted in PBS with 1 % bovine serum albumin, they were incubated with avidin DH-biotinylated horseradish peroxidase H complex. Finally, the sections were developed for 3 minutes in a substrate solution containing 0.05 % diaminobenzidine tetrahydrochloride (DAB) and 0.01 % hydrogen peroxide, washed, and counterstained with hematoxylin, dehydrated, and mounted[20].

Image Analysis for Immunostaining Interpretation

An image analyzer computer system was used to examine the immunostained sections and determine the area percentage of the immune stain. Image assessment was carried out with a Leica microscope fitted with a digital video camera and software (Leica Qwin 500, manufactured by Leica Microsystems, Wetzlar, Germany). Positive immunolabeling was defined as a brownish color in the nucleus of the cells; the cellular reaction was assessed by calculating the percentage area of PCNA

immunoreaction in each specimen of the four groups using magnification (400x).

Statistical analysis

The whole collected data via computerized image analysis has been analyzed statistically. The values of the percentage area of PCNA immune expression were provided as mean and standard deviation (SD). The analysis of variance (ANOVA) test was used to compare the mean area percentage values of PCNA between groups. The data were coded and entered using version 26 of Statistical Package for the Social Sciences (SPSS) (IBM Corp., Armonk, NY, USA). The data were summarized using the mean and standard deviation. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc Tukey test. *P-values* less than 0.05 were used to determine statistical significance[21].

RESULTS

The Cur NPs Characterization

Imaging and analysis of Cur NPs with a transmission electron microscope (TEM) showed that the particles were spherical and averaged 19 nm in size (Figure 1).

Histological examination results

Group I (passive control): Examining the dorsal surface under the light microscope reveals the typical histological characteristics of the epithelium and the underlying lamina propria.

The dorsal surface of the tongue was regularly covered by conical shaped filiform papillae with tapered ends. Most of the papillae followed the same direction. Regular epithelial rete pegs were seen. The underlying lamina propria showed well-defined C.T papillae. Collagen fibers and tiny blood vessels could be found, which were often relatively thin. Most of the tongue comprised well-defined striated muscle fibers, which could also be seen beneath the lamina propria. These fibers were grouped in bundles that were crossing and running in different directions (transverse, vertical, and longitudinal) (Figure 2).

Group II (positive control): The treatment with Cur-NPs alone did not affect the typical structure of tongue tissues. The histological results demonstrated the typical histological characteristics of the epithelium and the underlying lamina propria as was displayed in the passive control group (Figure 3).

Group III (irradiated): The filiform papillae showed markedly atrophic changes and disturbing patterns where they appeared very short, thin, narrow, and had blunt apex compared to those of the negative control group. The epithelial covering of the dorsal tongue surface demonstrated atrophy, and a decrease in height with areas of very thin epithelium devoid of epithelial ridges was noticed. The keratin layer appeared thin, torn, and detached in some areas. The epithelial cells showed areas of architecture disruption where cells lost their normal cohesion, disturbed basal cell arrangement, and became crowded with deeply stained nuclei. The supporting basement membrane became almost straight. In the underlying lamina propria, C.T. papillae were irregular and markedly reduced and they may be disappeared completely in some areas. The collagen fibers were dissociated and degenerated. Muscle fibers were highly atrophied, lost their regular organization, and were widely spaced in some regions (Figure 4).

Group IV (irradiated+ treated): The dorsal surface of the tongue displayed aberrantly normal filiform papillae regarding their height and orientation. The keratin layer was variable in thickness along the examined sections. Rete pegs were somewhat regular in shape and size. The basal cell layer appeared intact, and other layers appeared nearly normal. Muscle fibers showed slight atrophy, and the spaces between them were reduced(Figure 5).

Immunohistochemical Results

Proliferating cell nuclear antigen (PCNA) Immunohistochemical detection in groups I and II, the basal cells on the dorsal tongue surface reacted strongly to PCNA immunostaining. Moreover, nuclear immunostaining moderately affected some cells in the lower portion of the prickle layer (parabasal). Few lamina propria cells displayed strong nuclear PCNA immunoreactivity (Figures 6 A,B). In group III, the dorsal surface epithelia showed an apparent reduction in the number of immunoreactive nuclei between basal cells with weak immunoreactivity. But the lamina propria cells displayed very weak immunoreactivity (Figure 6C). In group IV, the basal cells showed strong PCNA nuclear immunoreactivity, and some parabasal cells displayed moderate reaction. The cells of the lamina propria showed mild to moderate immunoreactivity (Figure 6D).

Statistical Results

Positive control (Cur NPs group) demonstrated the statistically highest mean area percentage of PCNA immunopositivity among all groups, which is shown in (Figure 7). Regarding group II (Cur NPs group) compared to the negative control group showed no significant difference with a *P-value* = 0.233. Group III (irradiated group) showed a significant reduction compared to the positive control and negative control groups, with a *P-value* of 0.001. Group IV (irradiated+ treated) demonstrated no significant difference compared to the negative and control group with a *P-value* of 0.826 and 0.071, while it showed a highly significant increase in the mean area percentage of PCNA immuno-positivity compared to the irradiated group with a *P-value*<0.001 which are presented in (Tables 1,2).

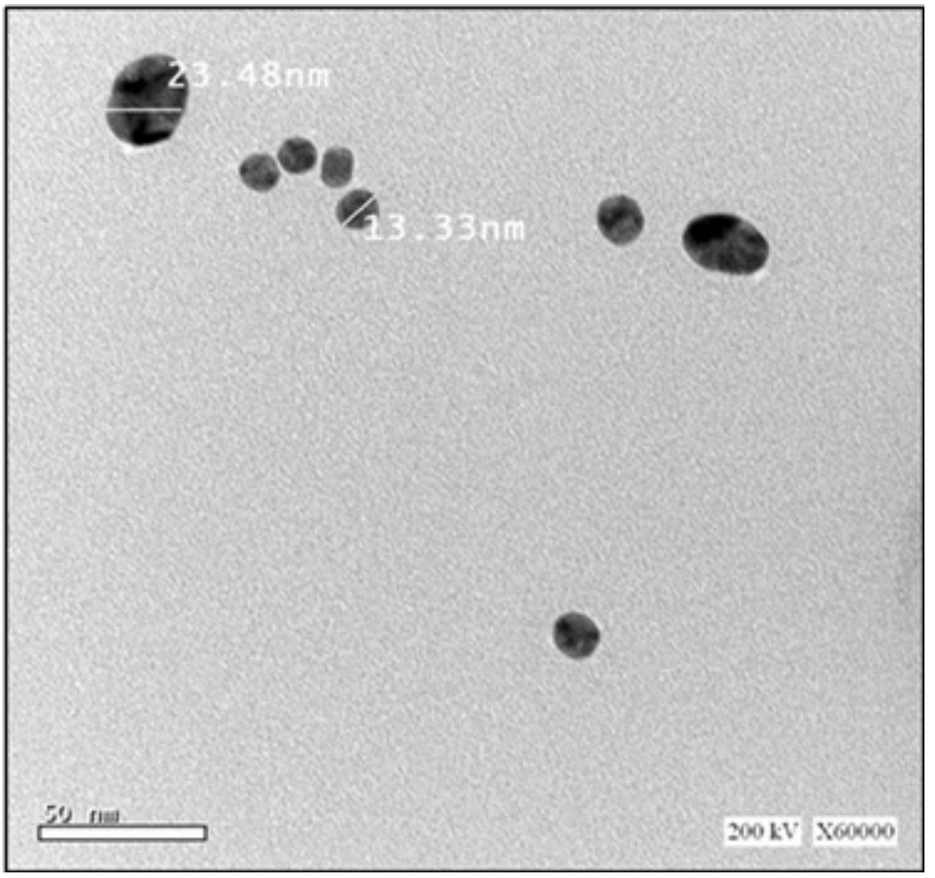

Fig. 1: TEM image showing Cur NPs characters (scale bar 50 nm)

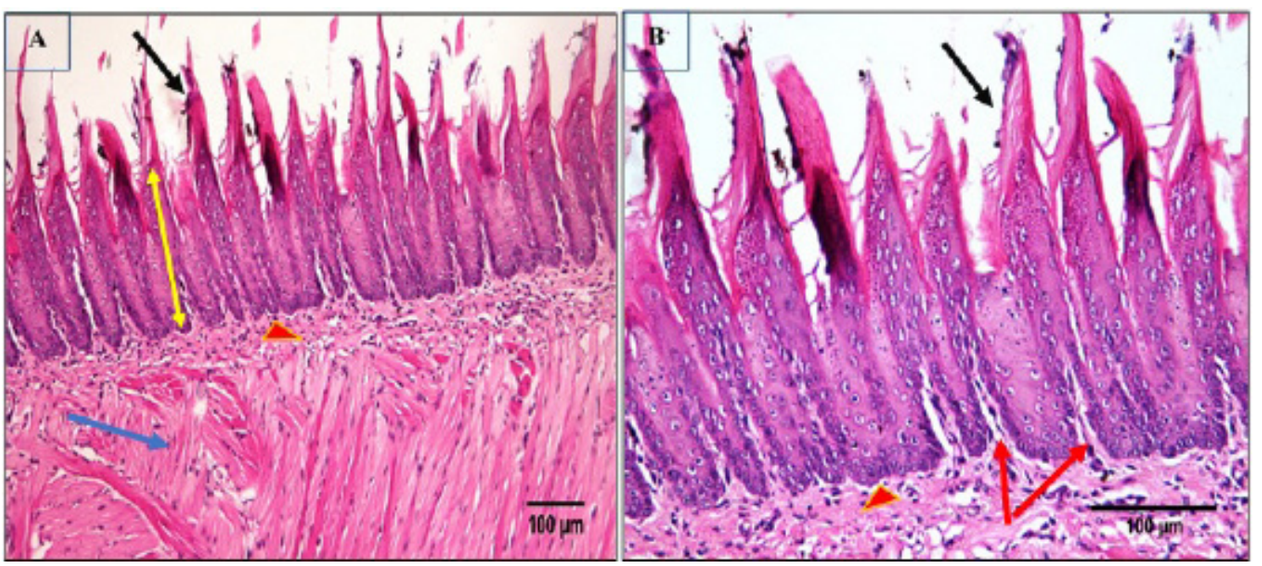

Fig. 2: a Photomicrograph of the dorsal surface of the tongue of group I showing conical, filiform papilla with an averaged epithelial thickness (yellow arrow), thickened keratin layer (black arrows), regular rete pegs (arrowheads), well defined C.T papillae (red arrows) and well organized muscle fibers (blue arrow). (H&E, Orig. Mag.(A) 200, (B) 400), Scale bar=100 µm).

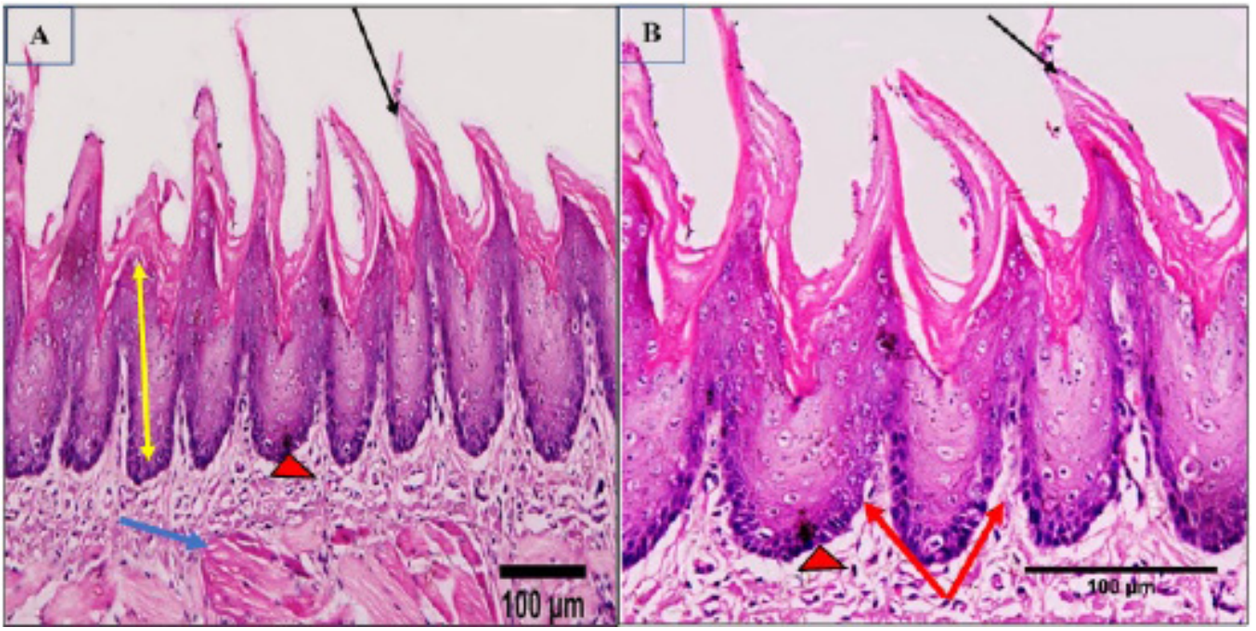

Fig. 3: a Photomicrograph of the dorsal surface of the tongue of group II showing the typical form of the filiform papilla, with an an averaged epithelial thickness (yellow arrow), thickened keratin layer (black arrows), regular rete pegs (arrowheads), well defined C.T papillae (red arrows) and well organized muscle fibers (blue arrow). (H&E, Orig. Mag.(A) 200, (B) 400), Scale bar=100 µm)

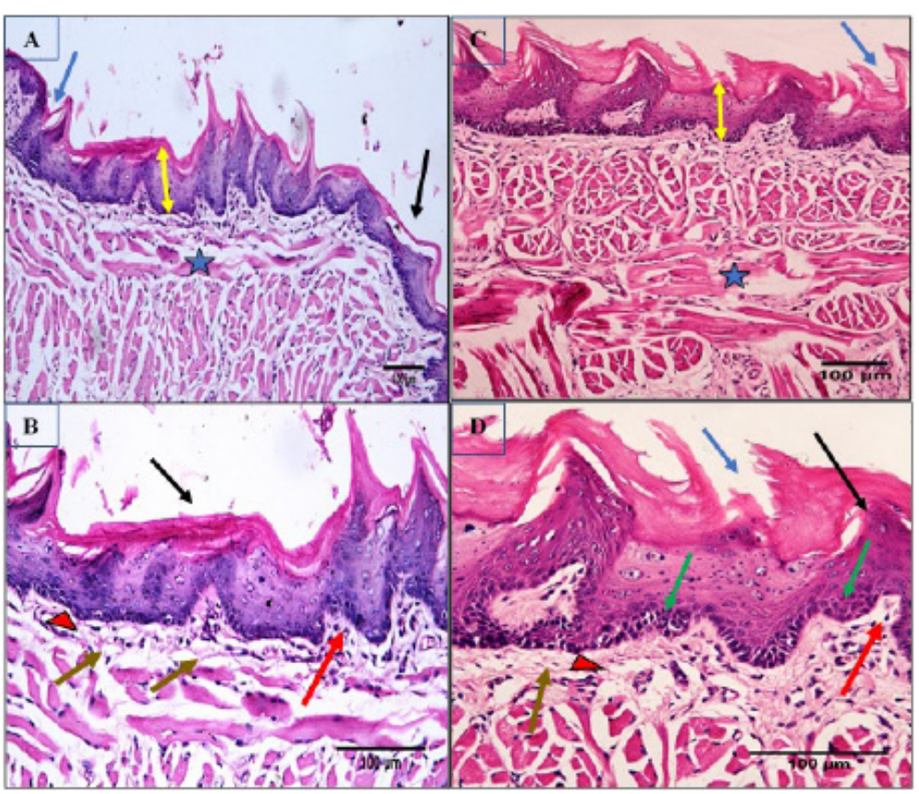

Fig. 4: a Photomicrograph of the dorsal surface of the tongue of group III showing atrophied filiform papilla (black arrows), thinning of the epithelial thickness(yellow arrows), torn and detached keratin layer (blue arrows), nearly straight B.M., devoid of rete pegs(arrowhead) and markedly reduced C.T papillae (red arrows), disturbed architecture of basal cells (green arrow), dissociation of collagen fibers (brown arrows), dispersed atrophied muscle fibers (stars) (H&E, Orig. Mag.(A),(C) 200, (B),(D) 400, Scale bar=100 µm).

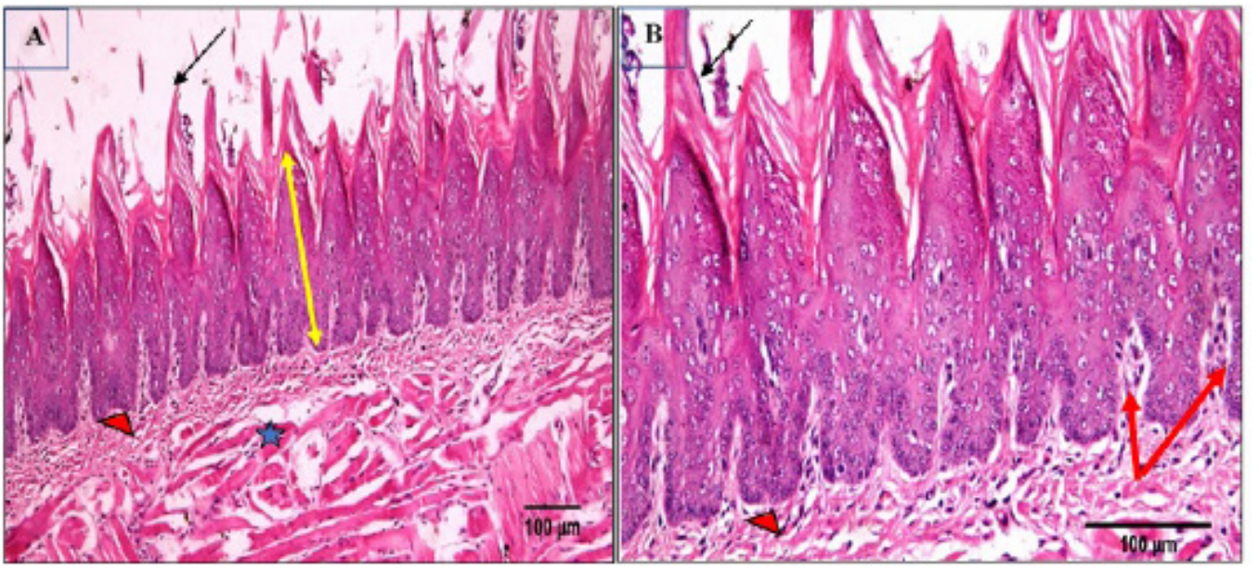

Fig. 5: a Photomicrograph of the dorsal surface of the tongue of group IV showing aberrantly regular filiform papillae (black arrows), averaged epithelial thickness (yellow arrow), standard epithelial rete pegs(arrowheads), and C.T papillae (red arrows), minimally dispersed atrophied muscle fibers (stars). (H&E Orig. Mag.(A) 200, (B) 400), Scale bar=100 µm).

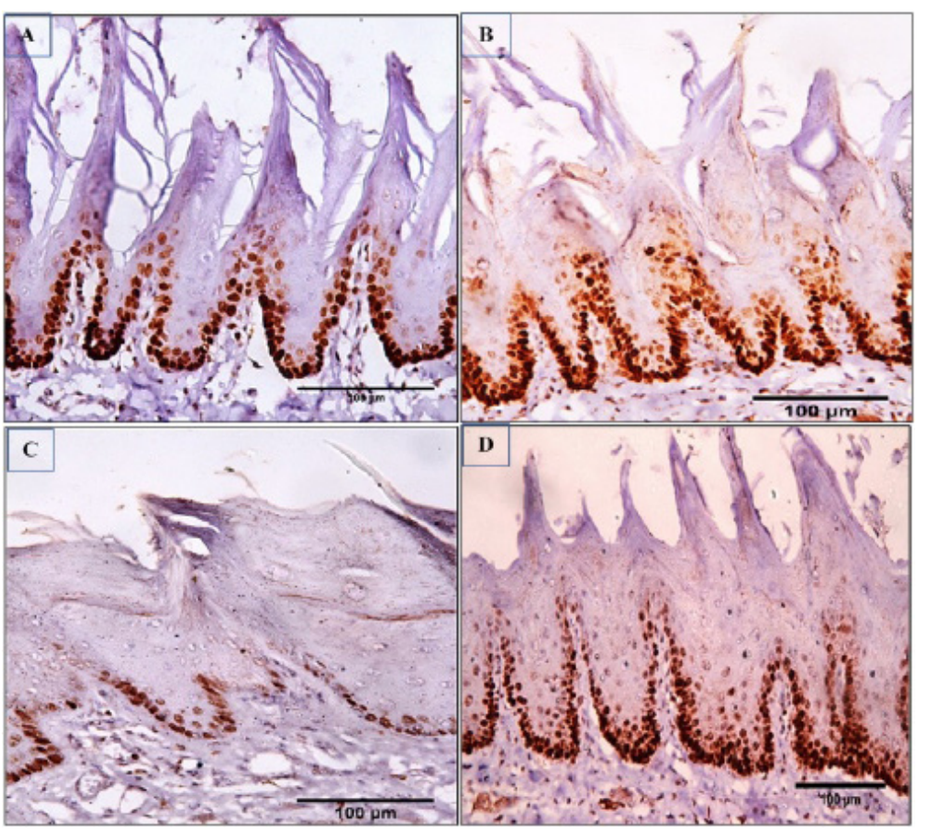

Fig. 6: PCNA Immunostained sections showing the reactional changes in the dorsal surface of the tongue tissues of all research groups. (A) Group I showing immunopositively stained all basal and some parabasal cell layers, less detectable positive reaction in the lamina propria. (B) Group II showing the same reactions of group I. (C) Group III showing negative to weak positive reactivity of the basal cell layers, negative reaction of the parabasal cell layers, weak immunoreactivity of the lamina propria. (D) Group IV showing immunopositively stained all basal and some parabasal cell layers, noticeable immunopositivity in the lamina propria (anti-PCNA, Orig. Mag. 400, Scale bar=100µm).

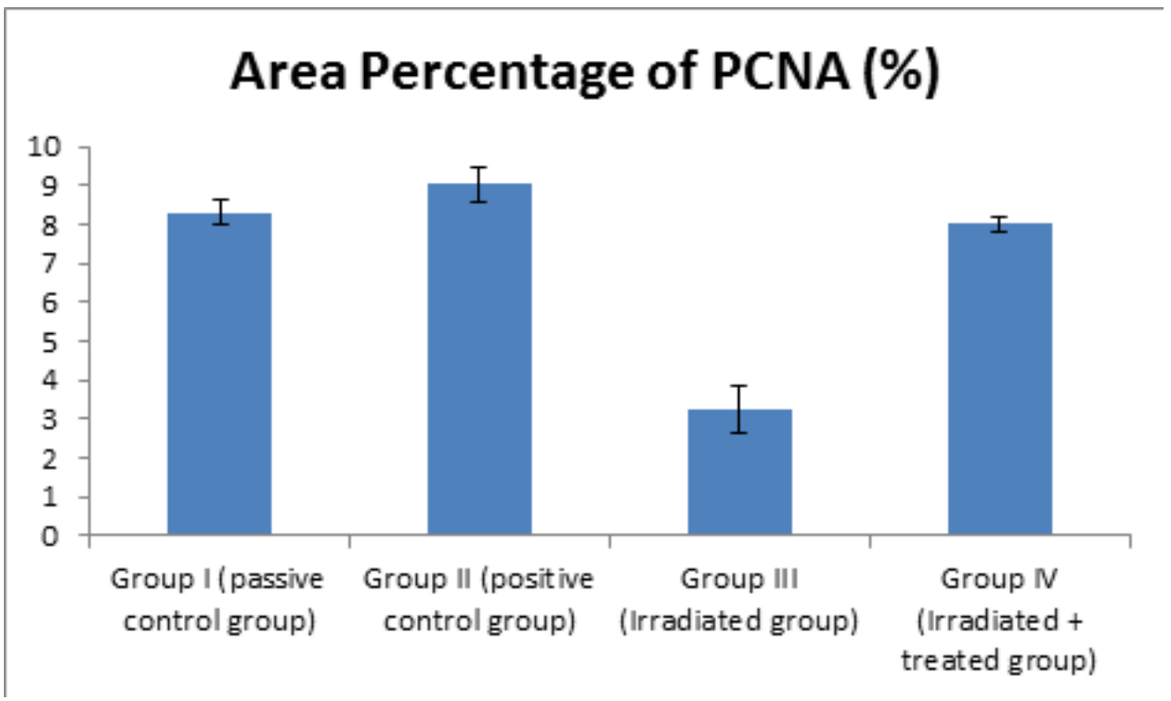

Fig. 7: Represents the difference in the mean area percentage of PCNA between all groups after one week

Table 1: Showing a comparison between the mean of anti-PCNA area percentage immunoreactivity between all groups after one week

	Group I (passive control group)		Group II (positive control group)		Group III (Irradiated group)		Group IV (Irradiated + treated group)		*P value*
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Area percentage of PCNA (%)	8.307	0.334	9.028	0.433	3.242	0.613	8.013	0.184	<0.001

Table 2: Showing Post hoc pairwise comparison (P-value between every two groups)

	Group I (passive control group)	Group II (positive control group)	Group III (Irradiated group)
Group II (positive control group)	0.233		
Group III (Irradiated group)	<0.001	<0.001	
Group IV (Irradiated + treated group)	0.826	0.071	<0.001

DISCUSSION

Tongue ulceration is a type of mucosal damage with high-grade oral mucositis seen in cancer patients treated with radiotherapy[22]. The current study aimed to identify possible changes in tongue mucosal tissues caused by locally applied radiation and the therapeutic potential of Cur- NPs in the albino rat model. The harmful impacts of ionizing radiation on mucosal tissues are secondary to ROS generation, and inflammatory mediators and cytokines release. These results activate various signaling pathways in the epithelium and sub-epithelial tissues, inhibiting epithelial cell renewal, promoting apoptosis, increasing atrophy, and causing ulcers[23]. Controlling O.M. is crucial for a better prognosis and overall quality of life. Many different agents have been investigated as potential preventative measures or therapeutic options for O.M., with a recent focus shifting towards natural-based compounds due to their lower potential for side effects compared to chemical products.

Additionally, animal models have produced a wealth of information regarding the processes of radiotherapy-induced mucosal injury and the anti-inflammatory benefits of nanoparticles derived from natural sources. The tongue model was chosen because it has the highest reproducibility, is easy to use, allows for a broader irradiation area, and provides adequate tissue sampling.

The stratified squamous epithelium that makes up the oral mucosa is a tissue that is constantly regenerating. To

balance desquamated cells from the surface layer, epithelial stem cells in the basal germinal layer multiply at a high renewal rate. While the mucosa's superficial layers continue to lose cells after radiation treatment, the deeper basal cells are harmed by radiation and cannot restore the lost cells. As a result, the mucosa thins out, and a broken mucosa eventually forms once the number of epithelial cells hits a critical point[24]. This study's results of the irradiated group revealed histopathological changes in epithelial tissues of the dorsal surface, including reduced epithelial thickness, a disturbing pattern of filiform papillae, apoptotic cells, and disturbed basal cell arrangement. These findings agreed with Watanabe *et al*.[25], who stated that mucosal injury induced by radiotherapy is caused due to the production of ROS in basal squamous epithelial cells. They cause DNA damage, followed by apoptosis and the formation of inflammatory cytokines, which results in the injury of the basal epithelial cell and subsequent death. Consistent with the outcomes of Zhao *et al*.[26], who noted thinning of the mucosa on the ventral tongue surface four days after radiation exposure in mice. Additionally, Li *et al*.[27] created a rat model of RT-induced oral mucositis in which ulcerative lesions, necrosis, and exfoliation of squamous cells all happened after 7 to 15 days. Furthermore, Tobita *et al*.[28] found that radiation reduced the ability of the cultured cells to proliferate, with only a small number of cells being seen in mitosis, ultimately leading to a reduction in epithelial thickness in an in *vitro* study. Curcumin has several biochemical functions, including stimulating cell proliferation and preventing apoptosis; it serves a vital role in maintaining epithelial and tissue integrity, as well as its essential function as an antioxidant in the defense against damage caused by free radicals through inflammatory reactions, which protect keratinocytes and fibroblasts from hydrogen peroxide-induced oxidative damage[29,30]. Additionally, research has demonstrated that curcumin has anti-cancer and radioprotective actions[31]. Nanoparticles have been used as nano-antioxidants, many of which have shown potential utility in nanomedicine. These nanoparticles are thought to be more effective and useful in countering free radical damage and provide an innovative tactic for antioxidant therapy to treat and prevent disease[32]. According to research, Cur-NPs increase curcumin's solubility, biological half-life, and tissue distribution in preclinical animal models to enhance its therapeutic effects[33]. Cur-NPs had stronger anti-inflammatory and antiproliferative effects in the examined animals than native curcumin, according to research by Dende *et al*.[34]. Additionally, many in *vitro* and in *vivo* investigations highlighted that Cur-NPs outperformed native curcumin in treating various disorders, including liver damage[35]. Cur-NPs have improved their pharmacokinetic properties by showing a considerable increase in bioavailability compared to plain curcumin. Furthermore, when utilized in human clinical trials, Cur-NPs demonstrated 100 times higher bioavailability than regular curcumin[36]. So, the choice of oral administration Cur-NPs in this study was preferred to provide high bioavailability. It was agreed with Hani & Shivakumar[37]; Sasaki *et al*.[38], who have documented that curcumin's low bioavailability has been identified as a major constraint for its pharmacological effects. According to a pharmacokinetic study, curcumin nanoparticles have a significantly higher oral bioavailability than curcumin administered with an absorption enhancer[39]. The histological picture in the rats' tongues treated with Cur NPs (group IV) revealed that Cur NPs are capable of restoring integrity, decreasing the severity of mucositis, and enhancing recovery when compared to the irradiation group by a growth in the epithelium thickness and the keratin covering. This histologic improvement in this study supports the results of Mosa *et al*.[40], who found that administering Cur NPs reduced TNF and IL-6, improved phagocytosis extent, and killed cell biological activity in gastric tissues. Moreover, in head and neck squamous cell carcinoma cell lines, Chang *et al*.[41] revealed that curcumin-loaded nanoparticles significantly inhibited cell growth while also inducing apoptosis in the cancerous cells. According to Shabeeb *et al*.[42]. In the study, administering curcumin to rats' skin before and after radiation reduced radiation-induced oxidative damage and boosted their antioxidant defenses. This study used PCNA, a cell cycle-related nuclear protein most abundant in proliferating cells during the late G1 and S stages. The expression of PCNA is directly correlated with cell division and DNA synthesis rates, therefore serving as a reliable marker of epithelial renewal[43]. The statistical findings of this study revealed that comparing all groups, the Cur NPs group demonstrated the statistically significant highest mean area percentage of PCNA immuno-positivity, which means Cur NPs stimulates the proliferation of cells and subsequently improve healing. These results corroborated with Shehzad *et al*.[44], who demonstrated that curcumin controls cell proliferation and progression through the cell cycle by regulating the action of numerous kinase proteins, cytokines, growth factors, and their receptors. The Food and Agriculture and the World Health Organizations advise consuming 0-1 mg/kg of curcumin per day, and numerous clinical studies have proved that it is safe and allowed even at doses up to 12 g/day.

PCNA expression was noticeably elevated in group IV after radiation than in the irradiated group; the administration of Cur NPs before and after the radiation may lead to a decreased degeneration of the tongue tissues. Cur-NPs can exert radioprotective effects in normal cells and stimulate epithelial cell proliferation is an intriguing feature of their action. These results agree with those of Aktas *et al*.[45], who examined the effects of curcumin's proliferative and anti-apoptotic properties on the mice ovarian follicles subjected to radiation. Hence, mice given Cur-NPs experienced a substantial rise in PCNA expression; radiated groups had a noticeably lower density in their granulosa cells. Furthermore, according to Kulac *et al*.[46], curcumin significantly enhanced PCNA expression in the skin tissues and re-epithelialization in the burn subgroup treated with curcumin compared to the burn subgroup in all groups.

CONCLUSION AND RECOMMENDATION

The current research outcomes highlighted that exposure to gamma radiation during radiotherapy has damaging and degenerative effects on the tongue tissues of rats and also revealed that Cur-NPs have a highly effective, protective, and regenerative role against the harmful effects of irradiation. Cur-NPs' efficacy in therapeutic interventions as a treatment approach for the protection and treatment of various irradiated tissues should be verified by more research and human trials.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التأثير الوقائي المحتمل لجزيئات الكركمين النانونية على اللسان المشع للجرذان الذكور البيضاء (دراسة هستولوجية وهستوكيميائية مناعية)

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الخلفية: على الرغم من أن العلاج الإشعاعي هو العلاج الأكثر استخدامًا للأورام الخبيثة في الرأس والرقبة، إلا أن معظم المرضى يعانون من مشاكل في الفم ناتجة عن تلف الحمض النووي وإنتاج أنواع الأكسجين التفاعلية اللاحقة (ROS). تلقى الكركمين، وهو أحد مضادات الأكسدة الموجودة في المستخلصات النباتية، تركيزًا مؤخرًا على فعاليته المحتملة ضد الاضطرابات المرتبطة بالإجهاد التأكسدي، خاصة عند استخدامه في الجسيمات النانوية. اللسان هو أحد الأعضاء الحيوية التي يمكن أن تتأثر بالآثار الجانبية للإشعاع. وتهدف هذه الدراسة إلى تقييم التأثيرات السامة للإشعاع على أنسجة اللسان المخاطية وتقييم التأثير الوقائي المحتمل لجسيمات الكركمين النانوية (Cur NPs) على هذه الأنسجة المشعة.

المواد والطرق: تقسيم ستة وثلاثين فأرًا أبيض ذكرًا بشكل عشوائي إلى أربع مجموعات، والمجموعة الأولى (التحكم السلبي) لم تتعرض للإشعاع ولا العلاج، والمجموعة الثانية (التحكم الإيجابي) تلقت الجسيمات النانوية الحالية دون إشعاع، والمجموعة الثالثة تلقت العلاج الاشعاعي فقط (المشعة)، والمجموعة الرابعة (الجسيمات النانوية المشعة+ الجسيمات النانوية الحالية). تعرضت رؤوس الفئران في المجموعتين الثالثة والرابعة إلى ١٥ غراي من الإشعاع. وتلقت الفئران في المجموعة الرابعة الجسيمات النانوية الحالية عن طريق الفم قبل ٤٨ ساعة من الإشعاع ويومياً (١٠٠ ملغ/كغ) لمدة أسبوع. في نهاية التجربة، تم تشريح أنسجة اللسان ومعالجتها للتحليل النسيجي والكيميائي الهيستولوجي المناعي.

النتائج: أظهر الفحص النسيجي للمجموعة الثالثة تغيرات مدمرة في أنسجة اللسان، لكن المجموعة الرابعة أظهرت إعادة تكوين انسجة اللسان بشكل أكبر بظهور سمات نسيجية الأقرب الى النسيج الطبيعي.

أثبتت النتائج الكيميائية النسيجية المناعية باستخدام مضادات PCNA أن الجسيمات النانوية الحالية أظهرت معدل تكاثر محسن للخلايا، مما يكشف عن تأثير شفاء أفضل في المجموعة الرابعة مقارنة بالمجموعة الثالثة.

الخلاصة: أنتج العلاج الإشعاعي تغيرات مرضية نسيجية وتلف في الأنسجة المخاطية في اللسان. ومع ذلك، فقد ثبت أن إعطاء الجسيمات النانوية قبل الإشعاع وبعده يقلل من هذه الآثار الضارة من خلال خصائصها القوية كمضادة للأكسدة وامضادة للالتهابات والمناعة.