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IMMUNOMODULATORY EFFECTS OF CURCUMIN COMBINED WITH PIPERINE ON INDUCED CARCINOGENESIS IN HAMSTER BUCCAL POUCH

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Abstract

Objective: Curcumin (Cu) exhibits antitumor and anti-inflammatory activity in oral carcinogenesis but has limited clinical translation due to poor bioavailability, which piperine (Pip) can improve. This study evaluated the antitumor and immunomodulatory effects of intraperitoneal Cu-Pip therapy, given for 2 or 4 weeks, in a DMBA-induced hamster buccal pouch carcinogenesis model, focusing on programmed death ligand-1 (PDL-1) and cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) expression. **Materials and methods:** Twenty male Syrian golden hamsters were allocated to a negative control (A1), a DMBA-only positive control (A2), and two DMBA-exposed groups treated intraperitoneally with Cu (80 mg/kg) and Pip (1 mg/kg) daily for 2 (A3) or 4 weeks (A4). Body weight, tumor area, leukocyte counts, serum IL-10/TNF-α, histopathological grade, and intratumoral PDL-1 and CTLA-4 expression were compared. **Results:** A3 and A4 groups showed significant reductions in body weight and tumor area relative to A2. Histopathology shifted from deeply invasive, moderately differentiated carcinoma in A2 to well-differentiated, superficially invasive carcinoma in A4. IL-10 and TNF-α were extremely significantly elevated in A2 relative to A1. TNF-α showed continuous elevation in A3 and A4, while IL-10 declined extremely significantly in A3 and A4 relative to A2. Neutrophilia and lymphopenia in A2 were partially reversed by treatment. CTLA-4 levels were extremely significantly reversed in A3 and fell extremely significantly in A4 relative to A2. PDL-1 declined significantly only in A4. **Conclusions:** Intraperitoneal Cu-Pip therapy reduced tumor burden and histopathological aggressiveness, with duration-dependent changes in the expression of cytokines and immune checkpoints. This suggests that there are immunomodulatory effects contribute to its antitumor activity, warranting further investigation.

Keywords: Oral Squamous Cell Carcinoma, Curcumin, Piperine, CTLA-4, PDL-1, Hamster Buccal Pouch.

1. INTRODUCTION

Oral squamous cell carcinoma (OSCC) accounts for the majority of malignant neoplasms of the oral cavity. It remains associated with substantial morbidity and mortality worldwide, especially in populations with high exposure to tobacco, areca nut, and alcohol [1].

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Despite advances in surgery, radiotherapy, and systemic therapies, survival outcomes for patients with advanced-stage disease have improved only modestly over the past decades. This limited progress is largely due to delayed diagnosis, extensive local invasion, and the ability of tumors to evade immune surveillance [2]. The etiology of OSCC is multifactorial, but chronic carcinogen exposure and the tumor-promoting inflammatory microenvironment accompanying sustained mucosal injury are recognized as the main drivers of malignant transformation [3]. The progression from normal mucosa to invasive carcinoma is increasingly understood not merely as a sequence of epithelial genetic alterations, but as a dynamic interaction between transformed cells and the surrounding immune microenvironment [4].

Cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10) participate in opposing arms of this interaction: TNF-α sustains chronic inflammatory signaling that can promote epithelial proliferation and genomic instability [5], whereas IL-10 exerts predominantly immunosuppressive effects that favor tumor escape from cytotoxic immune surveillance [6]. Concomitantly, tumor cells and infiltrating immune cells frequently upregulate inhibitory immune checkpoint molecules, including programmed death ligand-1 (PDL-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which restrain effector T-cell activity and contribute to an immunologically permissive niche for tumor growth [7] [8]. Systemic hematological alterations, such as neutrophilia accompanied by lymphopenia, have similarly been linked to tumor-associated immune dysregulation and are considered indirect markers of the host inflammatory-immune balance [9].

Curcumin (Cu), a polyphenolic compound derived from Curcuma longa, has demonstrated antiproliferative, antioxidant, and anti-inflammatory activity in numerous experimental models of carcinogenesis, including suppression of pro-inflammatory cytokine signaling and modulation of tumor-associated immune pathways [10]. Its clinical translation, however, has been limited by poor systemic bioavailability following oral administration, prompting interest in adjunct strategies to improve its pharmacokinetic profile [11]. Piperine (Pip), an alkaloid derived from Piper nigrum, inhibits hepatic and intestinal glucuronidation and has been shown to substantially increase the systemic bioavailability of co-administered Cu, providing a rationale for combined administration in experimental antitumor protocols [12]. Prior work using intraperitoneal Cu in a hamster buccal pouch (HBP) carcinogenesis model demonstrated measurable therapeutic efficacy against 7, 12-dimethylbenz[a]anthracene (DMBA) induced carcinoma [13].

Although the antitumor and anti-inflammatory effects of Cu have been documented in models of oral carcinogenesis, the extent to which a Cu-Pip combination modulates immune checkpoint expression, specifically PDL-1 and CTLA-4, within the tumor microenvironment has not been well characterized. The present study, therefore, aimed to evaluate the antitumor and immunomodulatory effects of intraperitoneally administered Cu combined with Pip, given for 2 or 4 weeks, in the DMBA-induced HBP model of oral carcinogenesis. Specifically, the study assessed tumor burden and histopathological grade alongside systemic hematological parameters, serum IL-10 and TNF-α levels, and

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the intratumoral expression of PDL-1 and CTLA-4, to determine whether treatment duration influences the pattern of immune checkpoint modulation. The hypothesis was that Cu-Pip treatment would reduce tumor burden and histopathological aggressiveness in a duration-dependent manner, and that this effect would be paralleled by measurable changes in the cytokine profile and immune checkpoint expression consistent with attenuation of tumor-associated immune evasion.

2. MATERIALS AND METHODS

The experiment was established after approval by the Ethical Committee and Faculty Council at the Faculty of Dentistry, Suez Canal University (number 709/2023). All study procedures were carried out in accordance with the recommendations outlined in the ARRIVE guidelines.

2.1 Experimental Design and Treatment Protocol

The experiment was conducted using 20 male Syrian golden hamsters (Mesocricetus auratus) weighing 90-120 g and aged 8-10 weeks. All animals were maintained on a standard laboratory pellet diet with free access to water and were allowed to acclimate to the housing conditions for two weeks before initiation of the study. One gram of DMBA (Chongqing Biospes Cat. No. BCS1807-1) was dissolved in 200 ml of heavy mineral oil (Sigma Aldrich, USA; Cat. No. 330760) to obtain a 0.5% DMBA solution. The left HBPs were painted with a camel hairbrush, number 4. The animals were allocated randomly into four main groups as follows: Group A1 (n=5) served as the negative control group (no DMBA painting). Group A2 (n=5) served as a positive control and was painted with DMBA (3 times/week) for 12 weeks. Both control groups were euthanized after 12 weeks. The remaining ten animals were painted with DMBA following the same protocol as group B, then subsequently divided into three treatment groups. Group A3 (n=5) was given an intraperitoneal injection of combined Cu (Glentham Life Sciences Cat. No. 458-37-7) (80 mg/kg) and Pip (Sigma Aldrich, USA. Cat. No. 94-62-2) (1mg/kg) (Sigma Aldrich, USA. Cat. No. 94-62-2) once daily for 2 weeks [13]. Group A4 (n=5) received the same treatment regimen for 4 weeks. Cu and Pip were dissolved in corn oil and administered at a consistent time each day.

All treated animals were euthanized the second day following the last treatment of the respective treatment periods using intraperitoneal injection with 100mg/kg thiopental sodium.

2.2 Specimens Collection

At the end of each treatment period, blood samples were obtained from anesthetized animals via retro-orbital puncture before euthanasia. Samples collected in EDTA-coated tubes were used for hematological analysis, including total white blood cell (WBC) count and differential leukocyte counts (lymphocytes, neutrophils, monocytes, and eosinophils). The remaining blood samples were centrifuged at 3000 rpm for 10 minutes to separate the serum, which was subsequently collected and stored at −20°C until ELISA analysis.

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2.3 Clinical Evaluation

At the end of each treatment period, the body weight of each animal was recorded before euthanasia. Gross examination of the HBPs was conducted to evaluate tumor development and size. Standardized high-resolution photographs were obtained and analyzed using FIJI ImageJ software to quantify tumor surface area. Images were calibrated using a reference scale, and tumor boundaries were manually outlined to calculate the area in mm^2. For specimens presenting multiple tumors, the cumulative tumor surface area was determined. All measurements were performed under standardized conditions, and the mean of three independent measurements was used for statistical analysis.

2.4 Enzyme-Linked Immunosorbent Assay (ELISA)

It was used to quantify cytokine levels in blood serum using commercially available Quantikine™ ELISA kits (Quantikine™, USA; Cat. No. D1000B) for IL-10 and (Quantikine™, USA; Cat. No. DTA00D) for TNF-α following the manufacturer's instructions.

2.5 Histopathological and Immunohistochemical Evaluation

After euthanasia, all the HBP tissues were excised, fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Serial sections (4–5 μm thick) were prepared and stained with hematoxylin and eosin (H&E) for histopathological examination. The stained slides were evaluated under a light microscope and digitally scanned to generate whole-slide images for further analysis. Tumor differentiation was graded according to the World Health Organization criteria for oral squamous cell carcinoma [14]. All evaluations were performed independently by two blinded oral pathologists to minimize observer bias and ensure consistency.

For immunohistochemical analysis, 5-μm paraffin sections were mounted on positively charged slides and stained with monoclonal anti-PD-L1 antibody (DAKO Denmark, Cat. No. SK006) and polyclonal anti-CTLA-4 antibody (ELK Biotechnology, Cat. No. ES8684). Using standard immunohistochemical protocols.

Following deparaffinization, rehydration, antigen retrieval, and blocking of endogenous peroxidase activity, sections were incubated with the respective primary antibodies, followed by horseradish peroxidase-conjugated secondary antibodies. Immunostaining was performed using the EnVision FLEX+ detection system (DAKO; Cat No. k8002).

Immunoreactivity was visualized using diaminobenzidine chromogen and counterstained with hematoxylin. The stained slides were examined microscopically, and representative images were captured using a high-resolution camera image analyzer system. Two blinded oral pathologists independently evaluated immunohistochemical expression to ensure assessment reliability and minimize observer bias.

Immunoreactivity was assessed quantitatively using FIJI ImageJ software to get the combined positive score (CPS) for PDL-1 [15]. The immune cell score (ICS) was used for

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CTLA-4 to quantify the percentage of positive immune cells within the intratumoral stroma [16].

2.6 Statistical Analysis

Descriptive statistics were generated using Microsoft Excel 365, and statistical analyses were conducted using Jamovi software (version 2.7.34). Data normality was assessed using the Shapiro–Wilk test. Results were expressed as mean ± standard deviation (SD). Differences among groups were initially evaluated using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test. A p-value < 0.05 was considered statistically significant, while a p-value < 0.01 was considered highly significant and a p-value < 0.001 was considered extremely significant.

3. RESULTS

3.1 Clinical Observations

Gross examination of the excised HBPs revealed distinct morphological differences among the experimental groups (Fig. 1). The pouches of negative control group (A1) displayed smooth, intact mucosa without any visible surface abnormality (Fig. 1A).

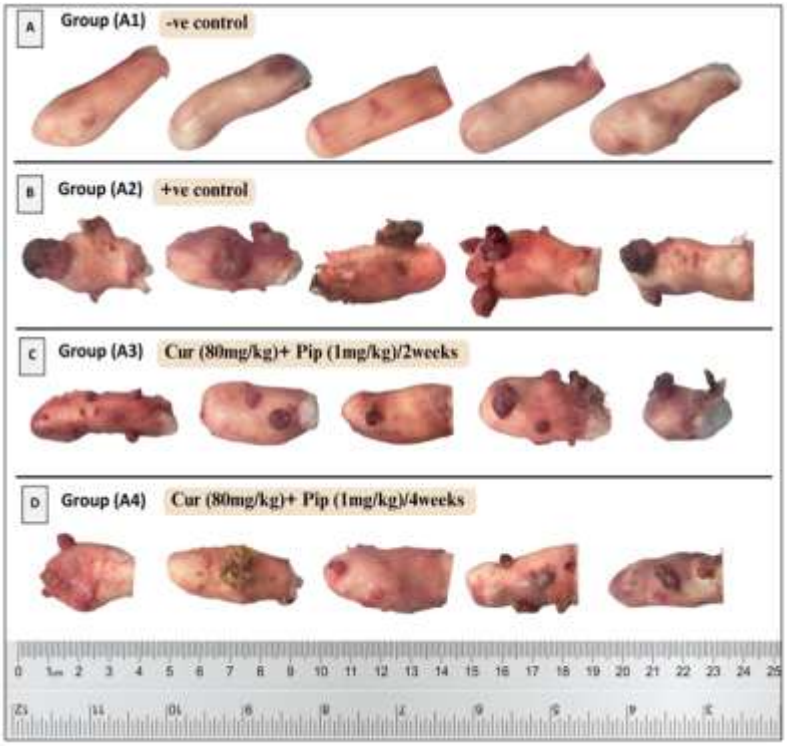

Figure 1: Representative Gross Morphological Appearance of Hbps from the Negative Control Group (A1), Positive Control Group (A2), And Cu with Pip Treatment Groups (A3 & A4). The Positive Control Group Exhibited Prominent Exophytic Tumor Masses, Whereas The Treated Groups Showed Reduced Lesion Size, Particularly After 4 Weeks of Treatment

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The painted left pouches of positive control group (A2) exhibited multiple exophytic, irregularly contoured tumor masses with a nodular, focally ulcerated and hemorrhagic surface, occupying a substantial portion of the pouch mucosa (Fig. 1B).

The left pouches of both Cu-Pip-treated groups (A3 and A4) showed a visibly reduced gross tumor burden relative to A2 (Fig. 1C& D). The mean of tumor area measurements in treatment groups A3 and A4 showed a highly statistically significant reduction compared to group A2 (Fig. 2A).

Body weights varied significantly between the different groups. Treatment group A3 showed highly significant weight reduction compared to control groups A1 and A2 ($p < 0.01$), while treatment group A4 showed extremely significant weight reduction compared to the A1 group ($p < 0.001$) and highly significant weight reduction compared to the A2 group ($p < 0.01$) (Fig. 2B).

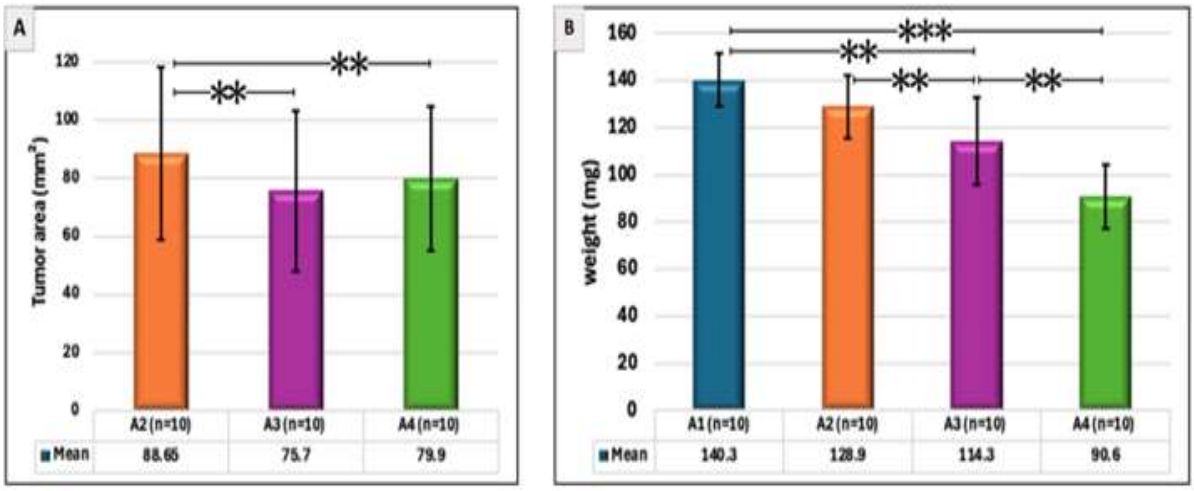

Figure 2: Animal Weight and Quantitative Assessment of Tumor Areai Control and Treatment Groups. (A) Tumor Area (Mm²) Comparison across the Groups. (B) Changes in Body Weight across the Groups. Statistical Analysis Was Performed Using One-Way ANOVA Followed By Tukey Post Hoc Test. **P < 0.01, ***P < 0.001. A1 (Negative Control Group), A2 (Positive Control Group), A3 (Cu & Pip / 2 Weeks), A4 (Cu & Pip / 4 Weeks)

3.2 ELISA Results

One-way ANOVA analysis demonstrated extremely significant intergroup differences in both IL-10 and TNF-α levels ($p < 0.001$). Group A2, A3, and A4 exhibited extremely significant elevation in IL-10 and TNF-α levels compared to the negative control A1. After 4 weeks of treatment, group A4 preserved the extremely significant elevation in TNF-α levels, but IL-10 levels exhibited extremely significant downregulation compared to the positive control group A2 (Fig. 3 A and B).

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3.3 WBCs Count Results

Total WBCs count was significantly elevated in the positive control group A2 ($p < 0.05$), while the

4-week-treated group A4 showed a highly significant elevation compared to the negative control group A1 ($p < 0.01$) (Fig. 3C). The differential leukocyte count showed severe neutrophilia and lymphopenia in group A2, while the Cu-Pip-treated groups (A3 and A4) showed partial restoration of lymphocyte count (Fig. 3D).

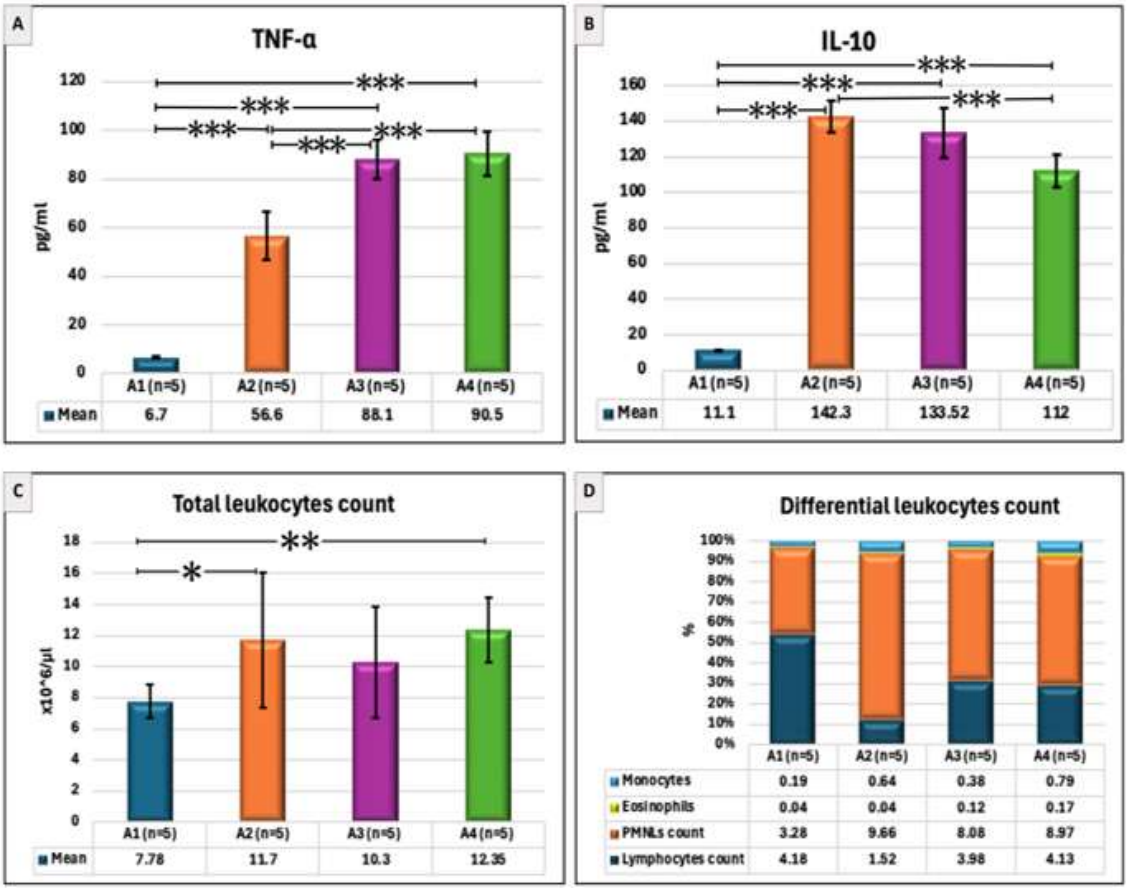

Figure 3: Serum Cytokine Levels And Leukocyte Count In Control And Treatment Groups. (A) TNF-A And (B) IL-10 Concentrations (Pg/Ml) Among The Groups. (C) Total Leukocyte Count And (D) Differential Leukocyte Distribution. Statistical Comparisons Were Made Using One-Way ANOVA Followed By Tukey Post Hoc Test. *P < 0.05, **P < 0.01, ***P < 0.001. A1 (Negative Control Group), A2 (Positive Control Group), A3 (Cu & Pip / 2 Weeks), A4 (Cu & Pip / 4 Weeks)

3.4 Histopathological Findings

Histopathological examination of H&E-stained sections demonstrated marked architectural differences among the experimental groups. The negative control group A1 exhibited the normal histological architecture of the HBP, characterized by a flat stratified

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squamous epithelium measuring two to four cell layers in thickness, devoid of rete ridges and covered by a thin keratin layer. The underlying lamina propria consisted of dense fibrous connective tissue, followed by a submucosal layer containing loosely arranged striated muscle fibers and a deeper loose areolar connective tissue layer (Fig. 4A).

In contrast, the positive control group A2 displayed exophytic deeply invasive, moderately differentiated squamous cell carcinoma (SCC) with pronounced infiltrative growth at the invasive tumor front (Fig. 4B). The 2-week treatment group A3 showed smaller exophytic lesions of well-differentiated SCC with moderate stromal invasion, indicating a reduction in tumor invasion depth compared with the positive control group (Fig. 4C). The 4-week treatment group A4 demonstrated further histopathological improvement, with predominantly smaller exophytic, well-differentiated SCC exhibiting only superficial stromal invasion. At the invasive front, the dysplastic epithelium formed broad, pushing rete ridges accompanied by a limited number of cohesive superficial epithelial islands (Fig. 4D).

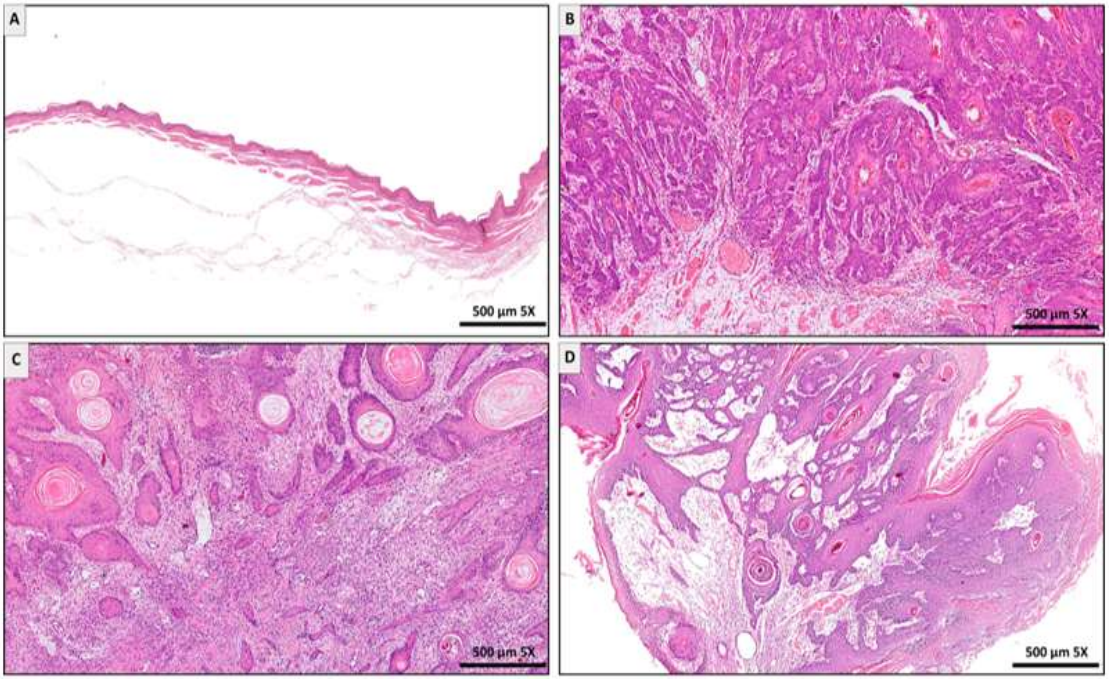

Figure 4: H and E-Stained Sections from the Negative Control, Positive Control, and Treated Groups. (A) The Negative Control Group A1 Showed Normal Histology Of The HBP, Composed Of 2-4 Layers Of Keratinized Stratified Squamous Epithelium Supported By Underlying Non-Inflamed Connective Tissue And A Mature Striated Muscle Layer. (B) Induced OSCC from the Positive Control Group A2 Demonstrates A Moderately Differentiated Exophytic Tumor Mass with Deeply Invasive Aggressive Infiltration. (C) The 2-Week Cu-Pip-Treated Group A3 Demonstrated Lesions of Moderately Invasive, Well-Differentiated OSCC with Reduced Depth of Invasion. (D) The 4-Week Cu-Pip-Treated Group A4 Exhibited Exophytic Lesions with Minimal Superficial Stromal Invasion

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3.5 Immunohistochemistry Results

Quantitative analysis of CTLA-4 expression of the immune cells in the 2-week-treated group A3 showed extremely statistically significant upregulation compared to the A2 group, whereas the 4-week-treated group A4 showed extremely significant downregulation compared to the A2 and A3 groups ($p < 0.001$) (Fig. 5A, B, C,&D). Quantitative analysis of PDL-1 (CPS) expression in epithelial tumor cells and stromal immune cells in the 4-week-treated group A4 showed statistically significant downregulation compared to the positive control group A2 ($p < 0.01$) and the 2-week-treated group A3 ($p < 0.001$) (Fig. 6A, B, C, &D).

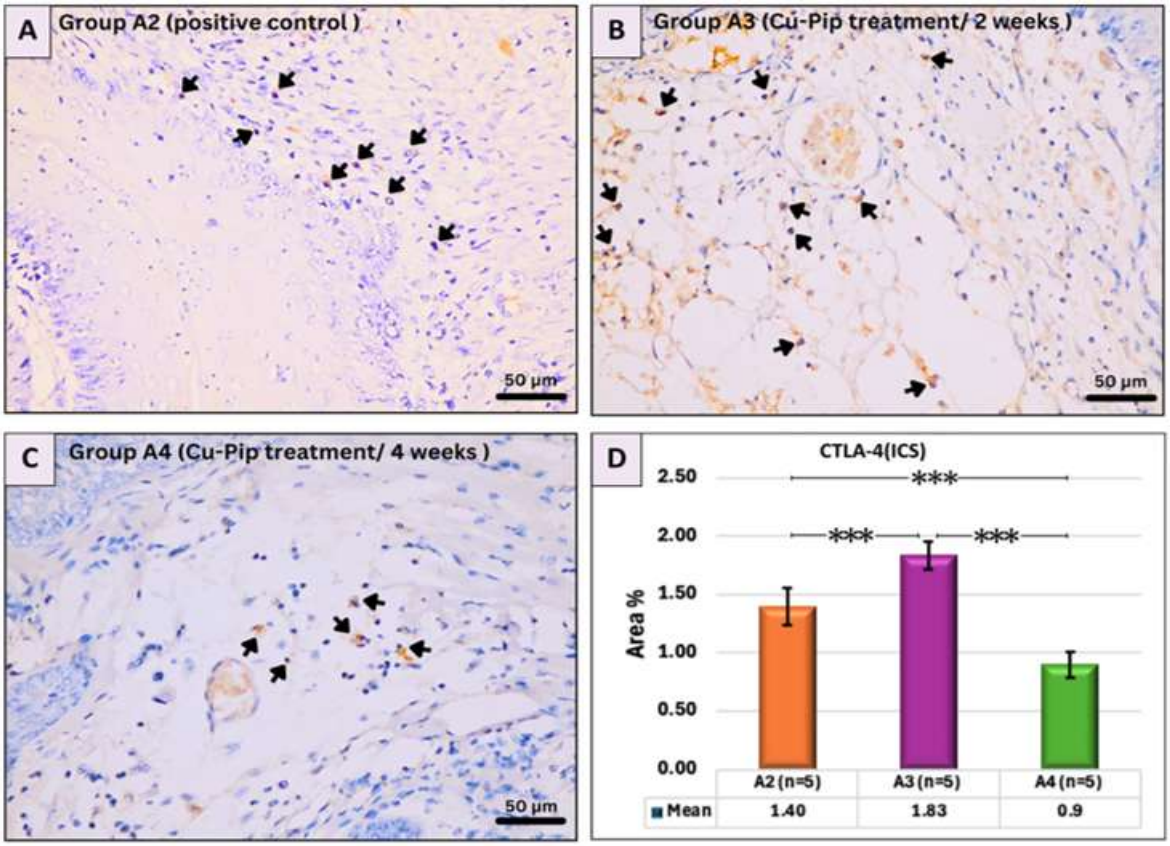

Figure 5: Representative Photomicrographs of CTLA-4 Immunohistochemical Staining In the Positive Control and Cu-Pip-Treated Groups. (A) Positive Control Group Showing CTLA-4 Immunoreactivity in Immune Cells (Black Arrows). (B) Cu-Pip-Treated Group (2-Week Treatment) Demonstrated Significant Upregulation Of CTLA-4 Expression. (C) Cu-Pip-Treated Group (4-Week Treatment) Showed Significant Downregulation. (D) The Bar Chart Shows The Mean Of CTLA-4 Expression And Pairwise Comparisons Between Groups. Statistical Comparisons Were Performed Using One-Way ANOVA Followed By Tukey Post Hoc Test. ***P < 0.001

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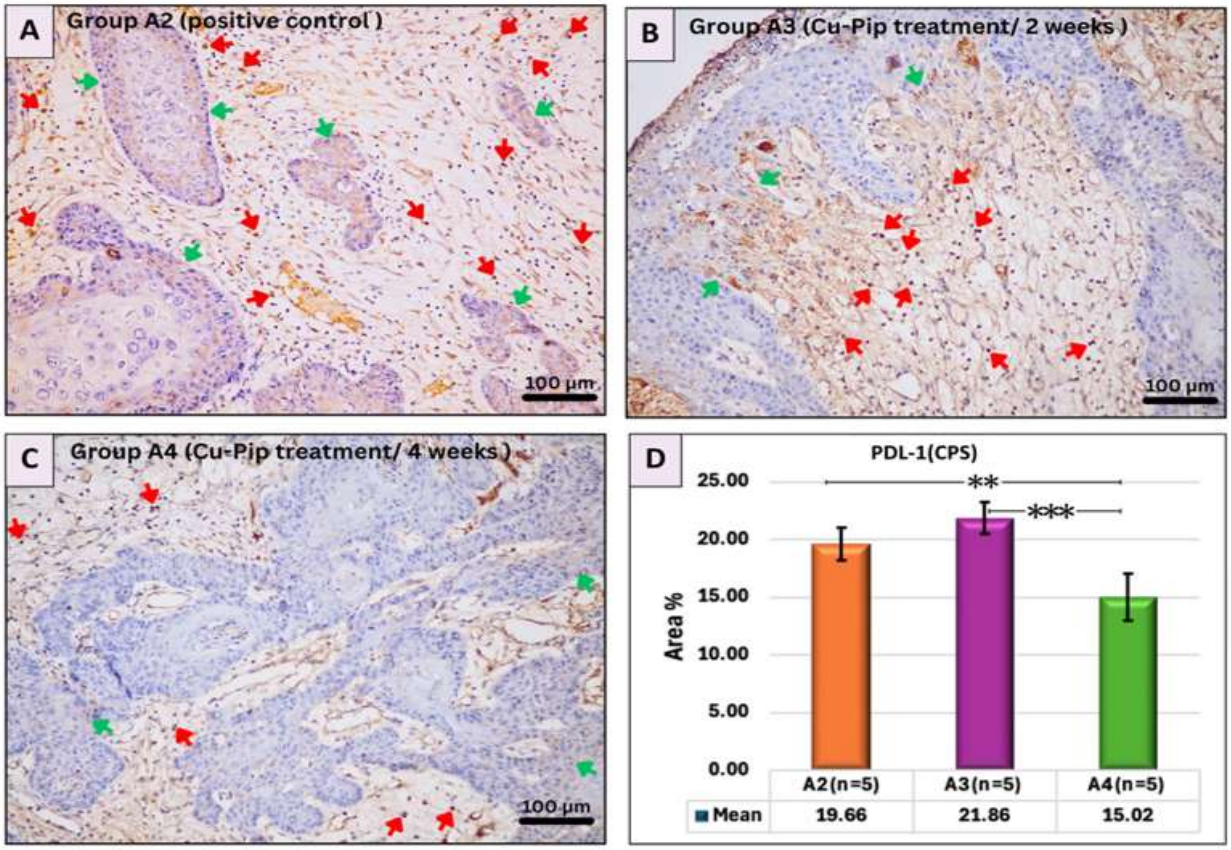

Figure 6: Representative Photomicrographs of PDL-1 Immunohistochemical Staining In the Positive Control and Cu-Pip-Treated Groups. (A) In The Positive Control Group A2, PDL-1 Immunohistochemical Staining Is Evident In Both The Epithelial Tumor Islands And The Surrounding Stromal Immune Cells. (B) PDL-1 Immunohistochemical Staining In the Two-Week Treatment Group A3, With the Emergence of Membranous Expression in Some Tumor Cells (Green Arrows). In Addition, Immune Cells Reactivity (Red Arrows). (C) PDL-1 Immunohistochemical Staining In Four Weeks of Treatment, With Significant Downregulation in Tumor Cells (Green Arrows) and Stromal Immune Cells (Red Arrows). (D) The Bar Chart Shows The Mean Of PDL-1 Expression And Pairwise Comparisons Between Groups. Statistical Comparisons Were Performed Using One-Way ANOVA Followed By Tukey Post Hoc Test. **P < 0.05, ***P < 0.001

4. DISCUSSION

The present study evaluated the antitumor and immunomodulatory effects of intraperitoneal Cu-Pip combination therapy administered for two and four weeks in the DMBA-induced oral carcinogenesis of HBP model. Treatment resulted in a reduction in tumor burden and a shift toward a less aggressive histopathological phenotype, changes that were accompanied by alterations in systemic leukocyte count, serum cytokine levels, and the intratumoral expression of the immune checkpoint molecules PDL-1 and CTLA-

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4. The direction and magnitude of several immunological changes differed between the two treatment durations.

The significant reduction in body weight observed in the treated group relative to the positive control is likely attributable to the cumulative effects of the underlying induced tumor and the treatment. As tumor-associated cachexia can be driven by chronic inflammatory cytokine release and increased metabolic demand in this model [17]. Superimposed on this pre-existing catabolic state, the pharmacokinetic properties of the treatment regimen may have further contributed to the significant weight reduction. Although Cu is generally considered to have a favorable safety profile when administered orally [18], the intraperitoneal route used in this study bypasses first-pass hepatic metabolism, resulting in higher systemic bioavailability than oral administration. The effect is likely amplified by Pip due to inhibition of hepatic and intestinal glucuronidation [12].

The significant reduction in tumor surface area in both treatment groups relative to the positive control is consistent with the antitumor efficacy previously reported for intraperitoneal Cu in this carcinogenesis model [13], and extends this observation to a Cu-Pip combination of the present study. The comparable magnitude of tumor area reduction at two and four weeks suggests that the known antiproliferative component of the treatment effect may be established relatively early in the treatment course, whereas, as discussed below, the immune checkpoints remodeling of the tumor microenvironment appears to continue evolving beyond this point.

The progressive histopathological improvement, from a deeply invasive, moderately differentiated carcinoma with an infiltrative growth pattern in the positive control group to a well-differentiated carcinoma with a pushing invasive front after four weeks of treatment, has important prognostic implications. A pushing tumor margin, in contrast to an infiltrative one, is generally associated with more favorable biological behavior [19]. The more pronounced histopathological improvement at four weeks compared with two weeks indicates that intratumoral immune remodeling continues over the course of treatment.

The extremely significant elevation of both IL-10 and TNF-α in the positive control group relative to the negative control is consistent with the chronic inflammatory state that characterizes DMBA-induced carcinogenesis, in which sustained cytokine release contributes to both tumor promotion and the establishment of an immunosuppressive microenvironment [20]. The significant decline in IL-10 after four weeks of treatment, without a corresponding decline in TNF-α, is of particular interest. IL-10 is a predominantly immunosuppressive cytokine that facilitates tumor immune escape by restraining effector T-cell and antigen-presenting cell function [21]. Its decline after prolonged treatment therefore suggests a partial reversal of this immunosuppressive signaling. The persistence of elevated TNF-α, in contrast, may reflect the dual role of this cytokine in tumor biology, whereby it can sustain inflammation-driven carcinogenesis under chronic low-grade conditions but also mediate cytotoxic and immune-activating effects in other contexts [22]. Within the present findings, sustained TNF-α elevation alongside reduced tumor burden and improved histopathological grade may be more consistent with an active antitumor immune response than with persistent tumor-promoting inflammation.

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The neutrophilia and lymphopenia observed in the positive control group are consistent with a systemic inflammatory response frequently associated with tumor burden and align with the broader literature linking altered neutrophil-lymphocyte dynamics to tumor-promoting inflammation and impaired antitumor immunity [23]. The partial restoration of lymphocyte counts in both Cu-Pip-treated groups suggests a shift away from this immunosuppressive hematological pattern, in parallel with the reduction in tumor burden.

The immunohistochemical findings for CTLA-4 revealed a biphasic pattern. Its expression increased significantly after two weeks of treatment relative to the positive control, then significantly declined by four weeks. CTLA-4 is expressed by activated and regulatory T cells and functions as a negative regulator of T-cell activation [16]. Its early increase could therefore reflect an initial influx or activation of T-cell populations within the tumor microenvironment as the immune system responds to treatment-induced tumor cell death or antigenic exposure, with CTLA-4 upregulation representing a compensatory checkpoint response to this heightened immune activity [24]. The subsequent decline by four weeks may correspond to a resolution phase, in which reduced tumor burden and antigen load diminish the stimulus for continued T-cell activation and checkpoint engagement [25].

In contrast to CTLA-4, PDL-1 expression, measured as the combined positive score across tumor and stromal immune cells, significantly declined only after four weeks of treatment, while it remained statistically unchanged relative to the positive control at two weeks. PDL-1 upregulation on tumor and stromal cells is a well-established mechanism of adaptive immune resistance consistent with elevated TNF-α in the treatment group A3, as TNF-α and interferon gamma-driven signaling in the context of an active antitumor immune response induces checkpoint ligand expression that subsequently suppresses effector T-cell function [26] [27]. The later significant decline in PDL-1 observed in group A4 may therefore represent a consequence of the immunological remodeling associated with prolonged treatment after the reduction in tumor burden [28]. The histopathological shift toward a less invasive phenotype and the decline in IL-10 and CTLA-4 had already become evident.

These findings raise the possibility that Cu combined with Pip may act not only as a direct antiproliferative agent but also as a modulator of tumor-associated immune checkpoint expression. Future studies should investigate a broader dose range and monotherapy arms to isolate the relative contributions of Cu and Pip. Extending the observation period beyond four weeks, together with survival analysis, would clarify whether the immunological remodeling described here continues, plateaus, or reverses with prolonged treatment. Additional characterization of tumor-infiltrating immune cell populations, including regulatory T-cell markers, alongside molecular analysis of relevant signaling pathways, would help establish the mechanistic basis for the observed changes in cytokine and checkpoint expression. Combination studies pairing Cu-Pip treatment with immune checkpoint inhibitors could also directly test whether the immunomodulatory effects described here translate into enhanced therapeutic efficacy.

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5. CONCLUSION

Within the limits of this preclinical model, intraperitoneal Cu-Pip combination therapy reduced tumor burden and promoted a less invasive histopathological phenotype in DMBA-induced HBP carcinogenesis. This antitumor effect was accompanied by a treatment-duration-dependent immunological response, including partial restoration of lymphocyte counts, decline in IL-10, and downregulation of CTLA-4 and PDL-1 expression that became significant after four weeks of treatment. These observations indicate that the therapeutic activity of the Cu-Pip combination is not limited to direct cytotoxic or antiproliferative effects but also may involve modulation of the tumor immune checkpoints.

Declaration of Interests

The authors declare that they have no competing financial or personal interests that could have influenced the results reported in this study.

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