

Bischofia javanica and *Phaleria macrocarpa* nano herbal combination on blood and liver-kidney biochemistry in Oral Squamous Cell Carcinoma-induced rats

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Abstract

This research explores a contemporary approach to managing Oral Squamous Cell Carcinoma (OSCC) by integrating nano-technology into herbal production, combining *Bischofia javanica* leaves and *Phaleria macrocarpa* fruits. Benzo[a]pyrene (BaP)-induced OSCC rats were treated with nano herbals from *Bischofia javanica* leaves, *Phaleria macrocarpa* fruits, a combination of both, and Vitamin C as a control. Blood and organ analyses were compared with negative and positive controls. BaP induction in OSCC revealed cell transformation into carcinoma, confirmed by Papanicolaou staining. OSCC induction also caused significant changes in Complete Blood Count (CBC) and serum lipid profile. Nano herbals from both plants showed potential in reducing hematological and lipid damage in OSCC, especially in white blood cells, red blood cells, hemoglobin, and platelets. Statistical analysis also indicated that the combination of nano herbals was more effective in reducing (Low-Density Lipoprotein) LDL and total cholesterol levels than uncombined use while increasing (High-Density Lipoprotein) HDL levels comparable to Vitamin C. Liver and kidney functions were also significantly affected by OSCC, with nano herbals showing potential in normalizing albumin levels and positively impacting liver enzymes. In conclusion, both individually and in combination, nano herbals from *Bischofia javanica* leaves, and *Phaleria macrocarpa* fruits have the potential to be a promising treatment strategy for OSCC.

Keywords

Oral Squamous Cell Carcinoma, nano herbal, *Bischofia javanica*, *Phaleria macrocarpa*

Introduction

Oral Squamous Cell Carcinoma (OSCC) poses a significant global health challenge. According to recent data from the International Agency for Research on Cancer (IARC) recorded in the Global Cancer Observatory, OSCC accounted for approximately 377.713 new cases

and 177.757 deaths in 2020 (Globocan 2020). These numbers signify a worrisome increase in incidence, emphasizing the need to develop contemporary approaches to managing OSCC. The pharmaceutical world has made significant strides in developing herbal materials in response to this demand. Recent innovations involve the integration of nano-technology in herbal production, as

demonstrated in this study by combining the leaves of *Bischofia javanica* and the fruit of *Phaleria macrocarpa*. This nano-approach offers advantages by enhancing the bio-availability and stability of herbal components, enabling more effective therapy with nano-sized elements that can improve penetration into cells (Arshad et al. 2021). Based on previous research, *Bischofia javanica* and *Phaleria macrocarpa* emerge as potential candidates for cancer treatment. *Bischofia javanica* is rich in bioactive compounds with antioxidant and anti-inflammatory activities, as well as phytochemicals such as alkaloids and terpenoids that exhibit anticancer potential through inhibiting cell proliferation and inducing apoptosis (Lingadurai et al. 2011; Lee et al. 2021; Susanto et al. 2022). Meanwhile, *Phaleria macrocarpa* presents bioactive compounds like polyphenols, flavonoids, and saponins, which have proven significant anticancer properties. Its antioxidant content can protect cells from oxidative stress, reduce the risk of DNA damage, and inhibit cancer cell mutations (Faried et al. 2007; Hasim et al. 2022). Its anti-inflammatory properties and potential as an immunomodulator also support its role in cancer treatment (Hendra et al. 2011; Kusmardi et al. 2019; Lee et al. 2021; Susanto et al. 2022).

This study evaluates OSCC-induced rats using Benzo[a]pyrene (BaP) and treated with a combination of nano herbals. Vitamin C is used as an alternative comparator to assess the effectiveness of nano herbal testing against OSCC. Vitamin C possesses strong antioxidant properties, which can help protect body cells from damage caused by free radicals. Free radicals can contribute to cancer development by damaging DNA and triggering abnormal genetic changes (Padayatty et al. 2003). Moreover, Vitamin C is also known to enhance the immune system, which can aid the body in fighting against the growth of abnormal cancer cells (Carr et al. 2017). Several studies have indicated that Vitamin C can directly affect cancer cells, including inducing apoptosis (programmed cell death) and inhibiting cancer cell growth. Vitamin C may also influence the efficacy of certain types of cancer treatments. Furthermore, Vitamin C is generally considered safe and rarely causes severe side effects (Villagran et al. 2021). In this research, the evaluation of the effects of nano herbal therapy and Vitamin C as a comparator on the physiological conditions of BaP-induced OSCC rats includes a comprehensive examination of blood profiles and organ biochemistry. In the context of OSCC, blood profile examinations can detect the presence of infection or inflammation through the differentiation of leukocyte types, such as neutrophils, lymphocytes, and eosinophils. Changes in the proportion of specific leukocyte types can indicate the response to OSCC or the impacts of the administered therapy (Garley et al. 2021). Meanwhile, organ biochemistry examinations, encompassing the levels of liver and kidney enzymes, offer insights into the integrity and function of vital organs.

Changes in enzyme activities or specific chemical levels can reflect the impact of OSCC on these organs or the side effects of treatment (Acharya et al. 2017). The importance

of monitoring blood profiles and organ biochemistry is also related to potential side effects or toxicity in therapy, especially in treatments involving herbal or nano-based compounds. These examinations can aid in identifying changes in side effects or potential toxicity that may arise during the treatment process (Ali et al. 2023). Additionally, by integrating this data, correlations between physiological changes and clinical responses to therapy can be observed, opening avenues for adjusting treatment plans based on individual patient responses to OSCC. Based on the above description, the objective of the study is to provide a detailed understanding of the potential therapeutic effects of the combination of nano herbal extracts from *Bischofia javanica* leaves and *Phaleria macrocarpa* fruit, offering valuable insights into the field of cancer research and nanomedicine, and exploring its potential as a promising intervention strategy.

Material and methods

Preparation of the nano herbals

The nano herbals utilized consist of *Bischofia javanica* leaf (NDS), *Phaleria macrocarpa* fruit (NMD), and a combination of *Bischofia javanica* leaf with *Phaleria macrocarpa* fruit (KSMD). Both plants were harvested from the local plantations in the Simalungun Regency, North Sumatra Province, Indonesia. The High Energy Milling (HEM) equipment produces these three nano herbals. The specific type of HEM utilized is the Emax High Energy Ball Mill, manufactured by Retsch. Although the processing for NDS, NMD, and KSMD is conducted separately, the methodology remains consistent. This method is generally adapted from the procedure outlined by Rumahorbo et al. 2023a. Before applying nano herbals from *Bischofia javanica* leaves and *Phaleria macrocarpa* fruit to rats with cancer, both nanomaterials undergo a series of toxicity tests, including cytotoxicity, acute toxicity, and chronic toxicity tests in the previous research. The LD50 value of *Phaleria macrocarpa* was $1 \pm 0,075$ g/kg BW, and the LC50 value was 2145,0407 ppm (Simanjuntak and Rumahorbo 2022). The LD50 value of *Bischofia javanica* was 12.6 ± 0.17 g/Kg BW, and the LC50 value was 3179.926 ppm (classified mildly toxic) (Rumahorbo et al. 2023a). For long-term use, the proper dose of *Phaleria macrocarpa* was 300–600 mg/kg BW (Rumahorbo et al. 2023b), and *Bischofia javanica* was 2–4 g/kg BW (Rumahorbo et al. 2023c). Additionally, the morphological and structural characterization of both nano herbals has been conducted through analyses such as Scanning Electron Microscopy (SEM) testing, Particle Size Analysis (PSA) testing, Fourier Transform Infrared Spectroscopy (FTIR) testing, phytochemical screening, antioxidant testing, and material characteristic evaluations, as previously explored in the research. The results can be observed in the following articles: Simanjuntak and Rumahorbo 2022; Rumahorbo et al. 2023a, 2023b, 2023c, and Rumahorbo et al., which is currently in press.

Animal handling and the treatment

This research involved 36 male rats (*Rattus norvegicus*) of the Wistar strain, weighing 180–250 g, and divided into six groups, each comprising six rats acclimatized for one week. Six rats without OSCC induction served as the negative control (K0). They were only given standard daily feed (Lab Diet - 5008 Formulab 23% Protein Rodent Diet). In comparison, the other 30 rats were initially induced with OSCC by injecting benzo[a]pyrene (Sigma-Aldrich Catalog number B1760) at 0.04 mg/0.04 ml corn oil (Brand Mazola) into the buccal mucosa of the proper oral cavity three times a week for four weeks. Each test subject underwent a swab test followed by a PAP smear (Papanicolaou Stain OG 6, Sigma-Aldrich Catalog Number HT40180) for Exfoliative cytopathology to ensure that the mucosal tissue had undergone differentiation towards carcinoma. Rats that tested positive for OSCC were grouped into five categories: K1 (Positive control), P1 (treated with 800 mg/kg/bw NDS), P2 (treated with 500 mg/kg/bw NMD), P3 (treated with 650 mg/kg/bw KSMD), and P4 (treated with 40 mg/kg/bw Vitamin C (Brand Allergy Research Group Pure Vitamin C 100 Vegetarian Capsules)). All doses were determined based on previous research described in the “Preparation of the Nano Herbals” section. All treatment substances were administered orally by suspending them in a 0.3% Carboxymethyl Cellulose Sodium (Brand Merck - 419273-1KG - Sodium Carboxymethyl Cellulose) solution once daily for four weeks. Blood samples were collected periodically due to the extensive requirements. A one-week interval separated the first and second blood collection batches. The handling of animals followed the technical specifications outlined in NOM-062-ZOO-1999 for the production, care, and use of laboratory animals, as well as complying with ARRIVE guidelines and the UK Animals (Scientific Procedures) Act, 1986, and related guidelines, and the EU Directive 2010/63 on animal experiments.

Exfoliative cytopathology with Papanicolaou (PAP) Smear

The procedure involved gently rubbing the mucosal surface in the suspected area using a cytology brush moistened with sterile physiological saline solution. The brush was then gently scraped onto a clean glass slide. This procedure was repeated up to three times, and the glass slides were subsequently placed in a staining dish containing Sifofix solution. This process was carried out for all test animals undergoing a PAP smear. Next, the samples of mucosal epithelium were stained with Papanicolaou (PAP Smear) stain and immersed in the staining solution for 30–40 seconds. After rinsing in running water and differentiation in 70% ethanol, the tissues were rapidly dehydrated through increasing alcohol concentrations up to absolute alcohol. The tissue slides were cleared in xylene, air-dried, and mounted using a mounting medium and coverslips. The evaluation was performed at 400x magnification, with suspicious cells being evaluated at higher magnifications. The

evaluation results were reported as a grading system, ranging from Grade 1 to Grade 5, indicating the severity of the cells. The grading of the observed preparations was based on the guidelines in the study by Sathawane et al. (2022).

Hematological and biochemical blood profile

Following the equipment's guidelines, blood samples from ethylenediaminetetraacetic acid (EDTA) tubes were tested in a hematology analyzer (Mindray Hematology Analyzer BC-700 Series). Hematological parameters measured included white blood cell count (Leukocytes), red blood cell count (Erythrocytes), hematocrit (HCT), hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Blood Cell Distribution Width (RDW), platelets (Platelets), neutrophils, lymphocytes, monocytes, eosinophils, and basophils. For the biochemical profile analysis, blood without EDTA was used. Blood samples were inserted into the analysis equipment. Parameters tested in the blood biochemical profile included Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), total cholesterol, and levels of albumin, globulin, complete protein, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), total bilirubin, creatinine, Blood Urea Nitrogen (BUN), uric acid, and blood sugar levels.

Statistical analysis

The research data were analyzed using Sigmaplot and Prism software. If the data met the normal distribution and variance homogeneity criteria, an ANOVA test was conducted at a significance level of 5%. If the ANOVA test results indicated significant differences ($p < 0.05$), the Post Hoc-Duncan test was carried out. a, b, c, and d represent significant group differences. In cases where the data did not meet the criteria for normal distribution and/or variance homogeneity, the non-parametric Kruskal-Wallis test was employed. For comparisons between the two treatments, the Mann-Whitney test was used. The symbols (*) indicate that the p-value is < 0.05 ; (**) for $p < 0.01$; (***) for $p < 0.001$; and (****) for $p < 0.0001$.

Result and discussion

Mucosal tissue analysis through exfoliative cytopathology with PAP smear

The count of the number of stained cells and their classification into the appropriate grade is recorded in Table 1. Statistical analysis revealed normal and homogeneous data distribution and the ANOVA test indicated significant differences in the overall data. Subsequently, a post hoc Duncan test was conducted to identify significant differences between groups.

Table 1. Gradation of mucosal cells in each group after PAP Smear.

Group	Grade I	Grade II	Grade III	Grade IV	Grade V
K0	64.6 ± 3.21 ^c	4.6 ± 3.51 ^a	2.2 ± 1.64 ^a	0 ± 0.00 ^a	0 ± 0.00 ^a
K1	2.2 ± 1.48 ^a	2.2 ± 2.59 ^a	5.2 ± 1.92 ^b	12.4 ± 3.05 ^b	62.2 ± 3.27 ^c
P1	37.8 ± 1.48 ^b	24.8 ± 2.64 ^c	15.4 ± 2.70 ^{bc}	8.8 ± 3.77 ^b	6.6 ± 4.51 ^b
P2	44.8 ± 4.38 ^c	21.8 ± 3.42 ^c	24.4 ± 3.65 ^c	3.5 ± 1.30 ^a	6.4 ± 3.78 ^b
P3	57.4 ± 3.97 ^d	24.4 ± 3.65 ^c	5.8 ± 3.77 ^b	2.8 ± 1.48 ^a	1.6 ± 0.89 ^a
P4	59.4 ± 2.07 ^d	15.4 ± 2.70 ^b	5.6 ± 3.91 ^b	4.2 ± 1.10 ^a	2.2 ± 0.84 ^a

Notes: a, b, c, and d represent significant group differences. The notation is interpreted by comparing the same grades in different groups. K0: Normal, K1: OSCC, P1: OSCC treated with nano herbal *Bischofia javanica*, P2: OSCC treated with nano herbal *Phaleria macrocarpa*, P3: OSCC treated with a combination of nano herbal *Bischofia javanica* and *Phaleria macrocarpa*, P4: OSCC treated with Vitamin C.

Duncan's post-hoc test showed that grade 1 in groups P3 and P4 showed no significant difference. (The same annotation, namely notation 'd') and only slightly between P3 and P4 with the control group (K0). This result indicates that the number of cells still classified as usual is significant in groups P3 and P4. The group with a cell grade distribution similar to group K1 was group P1. Compared to the two nano herbals in a single administration (not combined), the *Phaleria macrocarpa* nano herbal is better than the *Bischofia javanica* nano herbal in differentiating normal cells into carcinoma in oral mucosal tissue. Fig. 1 displays the results of observations on the buccal mucosa of the oral cavity suspected of OSCC after nano herbal therapy.

The mucosal tissue in K0 appears to have mild cell changes (regular and healthy epithelial cells). It has not led to cancer (Fig. 1A). The K1 group had a thick keratin layer, enlarged nuclei, nuclear polymorphism, hyperchromatic nuclei (darker color), and increased nucleocytoplasmic ratio. In addition, the cells in the image showed polarization (random and disorganized growth) (Fig. 1E). In group P3, the mucosa cells appeared to have less severe keratinization,

slightly enlarged cell nuclei, irregular shape, and an unbalanced ratio of nuclei to the cytoplasm (Fig. 1D). The condition of P3 was somewhat different from the P2 group (Fig. 1C). In the P1 group, the cells showed a histological picture similar to the K0 group, which was almost unobserved cell nuclei undergoing more significant changes and a more irregular cell shape (Fig. 1B). PAP analysis on oral preparations has a sensitivity of 91.176% and a specificity of 100%. The positive predictive value rate reached 100%, while the negative predictive value was 76.92% (Vyas et al. 2018).

Complete blood count analysis in OSCC-Induced rats treated with nano herbal therapies

Fig. 2 illustrates the statistical analysis results using ANOVA and post-hoc Duncan tests on data related to the complete blood count. The ANOVA results indicate significant differences between the control and OSCC groups in all hematological parameters, except for MCH and MCHC (Fig. 2), with a p-value < 0.01. BaP-induced OSCC leads to changes in the conditions of all measured blood parameters. This is evident from the different letters in the significant difference notation between K0 and K1 for all parameters, except MSH and MCHC (Fig. 2). However, when comparing treatment groups (P1–P4) with K0, some parameters show the same notation (for example, in the WBC parameter, P2 and P3 have the same notation as K0, namely notation 'bc,' but differ in notation between K0 and P1 and P4). To facilitate the interpretation of statistical analysis results for each analyzed parameter, compare each notation in each treatment group with the negative control group (K0). If the notation is the same, the therapy is effective because the measurement parameter values do not differ from the healthy group (K0).

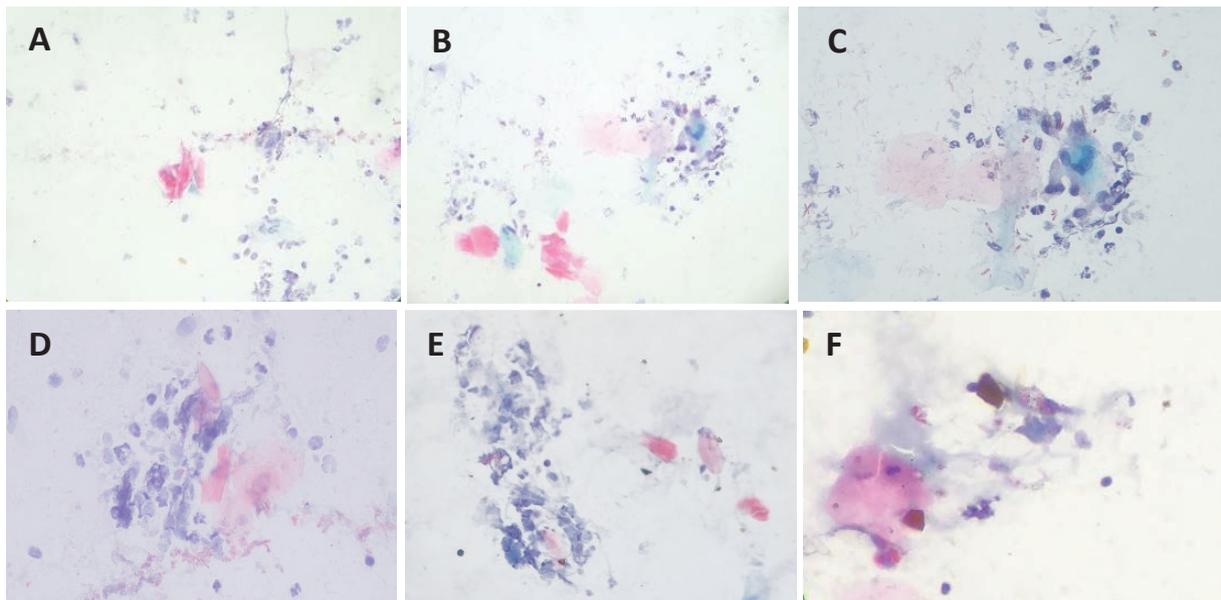


Figure 1. Pap Smear results of the oral mucosa of OSCC-Induced Rats; A. Grade I from K0; B. Grade II from P1; C. Grade III from P2; D. Grade IV from P3, E. Grade V from K1, and F. Grade I from P4. K0: Normal, K1: OSCC, P1: OSCC treated with nano herbal *Bischofia javanica*, P2: OSCC treated with nano herbal *Phaleria macrocarpa*, P3: OSCC treated with a combination of nano herbal *Bischofia javanica* and *Phaleria macrocarpa*, P4: OSCC treated with Vitamin C.

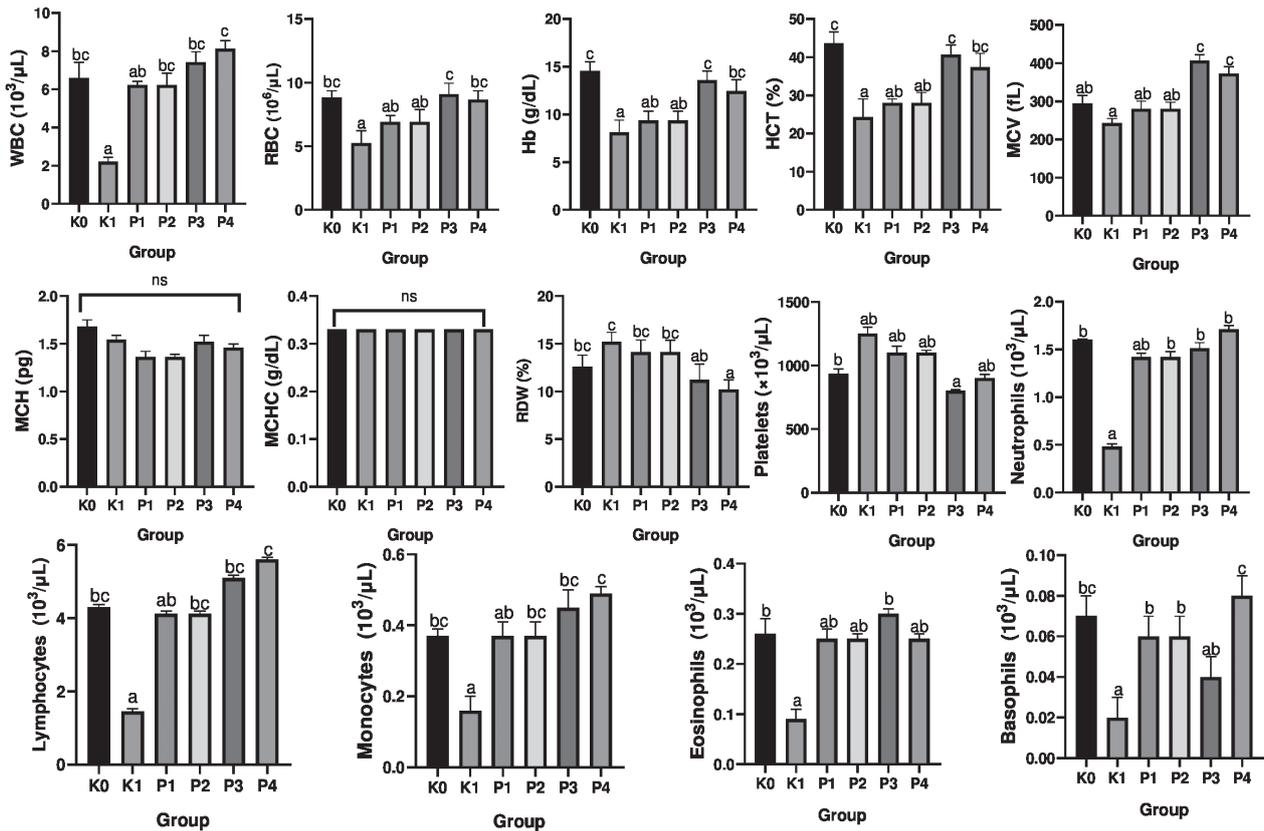


Figure 2. Hematological parameter values. a, b, c, d represent significant differences between groups. ns = $p > 0.05$ /Not substantial. K0: Normal, K1: OSCC, P1: OSCC treated with nano herbal *Bischofia javanica*, P2: OSCC treated with nano herbal *Phaleria macrocarpa*, P3: OSCC treated with a combination of nano herbal *Bischofia javanica* and *Phaleria macrocarpa*, P4: OSCC treated with Vitamin C.

Based on the findings of this study on hematological profiles, it is concluded that OSCC induction using BaP can lead to impairment in the physiological functions expressed through the complete blood count profile. An exciting aspect of this research is the condition of leukocytes. Certain types of cancers, such as leukaemia, lymphoma, and myeloma, can cause an increase in leukocyte count because these cancers develop in the blood system or bone marrow, where white blood cells are produced. However, in many non-blood-related cancers, there may not always be an increase in the white blood cell count. Conversely, some cancers can cause a decrease in the white blood cell count by inhibiting the production of normal white blood cells or causing damage to the organs that produce white blood cells (Giannakeas et al. 2022).

Although the body's response to cancer generally increases the leukocyte count, in the case of OSCC, specific factors can lead to a decrease in leukocyte count. OSCC can produce certain growth factors or cytokines that inhibit the production and function of leukocytes (Nguyen et al. 2020). In this study, inflammation occurring in experimental animals with OSCC can also affect the quantity and function of leukocytes. OSCC induction using BaP, a carcinogen, can disrupt white blood cells and decrease leukocyte count in the bloodstream (Nguyen et al. 2020). BaP induction plays a crucial role in disrupting the balance of blood components, spreading through blood vessels, and affecting white blood cells. Neutrophils, lymphocytes,

and monocytes, essential elements of the immune system, are influenced by excessive oxidative stress. Oxidative stress can also affect the production and function of platelets, which are critical in blood clotting. Hematological profiles are complexly affected, involving changes in red blood cells, white blood cells, platelets, and bone marrow (Pizzino et al. 2017; Bukowska et al. 2022). Nano herbals from *Bischofia javanica* leaves *Phaleria macrocarpa* fruit, and their combination can alleviate hematological damage caused by BaP induction. For instance, nano herbals from *Bischofia javanica* leaves effectively improve blood parameters such as leukocytes, erythrocytes, hemoglobin, hematocrit, MCV, RDW, and lymphocytes. Although *Phaleria macrocarpa* fruit appears to have a slightly better impact, this difference is not statistically significant (Fig. 2).

Serum lipid profile analysis in OSCC-Induced rats treated with nano herbal therapies

Fig. 3 illustrates the statistical analysis results using ANOVA and post-hoc Duncan test on data related to the profile lipid serum. Statistical analysis using ANOVA revealed significant differences ($p < 0.01$) between the control group and OSCC-induced groups across all serum lipid profile parameters (Fig. 3). Duncan's post hoc test confirmed significant differences in all serum lipid profile parameters between the negative and positive control groups, indicating that BaP

induction disrupts lipid metabolism. Notably, K0 showed substantial differences in LDL compared to K1, while K0 exhibited significant differences with other treatment groups, albeit with some overlap. Overall, both separately and in combination, nano herbal extracts from *Bischofia javanica* leaves and *Phaleria macrocarpa* fruit successfully reduced the elevated levels of LDL induced by BaP. However, the reduction was slight (notations a and ab, where notation a still overlaps). A significant increase was observed in total cholesterol (clearly distinct significant notations in each treatment group compared to K0). BaP induction led to a significant decrease in HDL in all groups compared to K0. Nano herbal therapy with *Bischofia javanica* leaves showed no significant change compared to K1, while *Phaleria macrocarpa* fruit induced a slight change with notation bc overlapping with ab in P2. P3 and P4 displayed substantial differences compared to K1 but coincided with K0.

Exposure to polycyclic aromatic hydrocarbons (PAH), such as BaP, disrupts various biochemical pathways, including lipid metabolism and cholesterol regulation, triggering oxidative stress and chronic inflammation (Bukowska et al. 2022). Monitoring LDL, HDL, and total cholesterol levels in OSCC is crucial for assessing the cardiovascular risk associated with oral cancer and its treatment. Nano herbal therapy can impact lipid metabolism, necessitating lipid profile monitoring to identify changes and plan appropriate responses, such as dose adjustments or additional treatment. Furthermore, lipid profiles are crucial in evaluating nutritional status, often affected by oral cancer. Thus, lipid measurements are essential for long-term monitoring in OSCC, aiding in understanding cardiovascular risks, managing nano herbal treatment side effects, monitoring nutritional status, and providing better care.

Liver and kidney function analysis in OSCC-induced rats treated with nano herbal therapies

Fig. 3 illustrates the statistical analysis results using ANOVA and post-hoc Duncan test on data related to the Liver and Kidney Function. Statistical analysis utilizing ANOVA revealed significant differences ($p < 0.01$) between the negative control group and the OSCC-induced group across all parameters of blood sugar and liver function (Fig. 4). Duncan's post hoc test revealed highly significant

differences in total bilirubin, creatinine, and blood urea nitrogen (BUN) parameters between the negative and positive control groups, indicating substantial liver damage due to BaP exposure. Notably, albumin levels showed similar results between the nano herbal-induced *Bischofia javanica* leaf group and the healthy control group, with no significant difference observed with the nano herbal-induced *Phaleria macrocarpa* fruit group. The negative control and nano herbal treatment groups exhibited no significant differences in total protein levels.

Meanwhile, liver enzymes AST, ALT, and ALP exhibited differences compared to the positive control group, with ALP showing similarity. On the other hand, BaP exposure significantly increased blood sugar levels. However, administering nano herbals from both plants, individually or in combination, and Vitamin C did not affect the elevated blood sugar levels induced by BaP. It's worth noting that the increase in blood sugar levels remained within normal limits based on *Rattus norvegicus* standards. This study contradicts previous research indicating that ethanol extract from *Bischofia javanica* leaves affects blood sugar levels (Hutahaean et al. 2021; Rumahorbo et al. 2021).

OSCC, as oral cancer, may indirectly influence liver function. Liver dysfunction in OSCC may be related to the systemic effects of the disease, such as inflammation and metastasis, rather than the direct involvement of cancer in liver function. The metastasis of oral cancer to the liver can directly impact liver function, but usually, oral cancer spreads to lymph nodes before affecting other organs. Therefore, the study results suggest that BaP-induced metastasis of OSCC may have reached the liver, affecting the biochemical profile of the liver measured from the blood and urine of OSCC-induced rats in this study.

Active cancer can induce metabolic stress, including in the liver, reflected in changes in the blood's albumin, globulin, total protein, and other parameters. Low albumin and total protein levels may indicate a decrease in liver protein synthesis. Although the primary reasons for low albumin levels in cancer patients are not fully understood, some mechanisms have been investigated. For instance, cancer cells can produce cytokines like interleukin-6 (IL-6), influencing albumin production. Cancer and inflammation-related processes can also affect liver enzymes such as ALT and AST, which increase in response to inflammation (Ando et al. 2021).

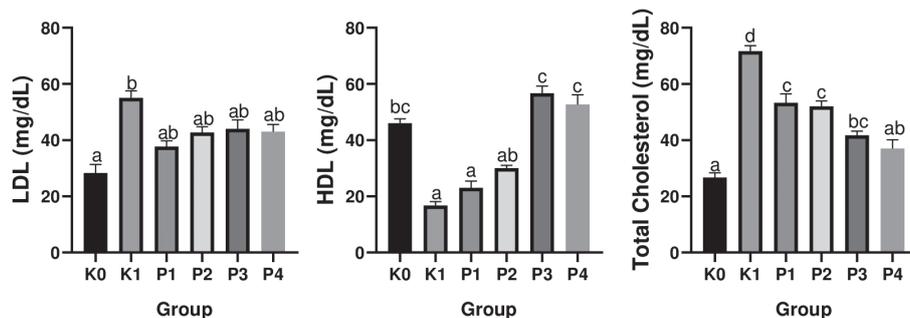


Figure 3. The serum lipid profile. Panels a, b, c, and d represent statistically significant group differences. K0: Normal, K1: OSCC, P1: OSCC treated with nano herbal *Bischofia javanica*, P2: OSCC treated with nano herbal *Phaleria macrocarpa*, P3: OSCC treated with a combination of nano herbal *Bischofia javanica* and *Phaleria macrocarpa*, P4: OSCC treated with Vitamin C.

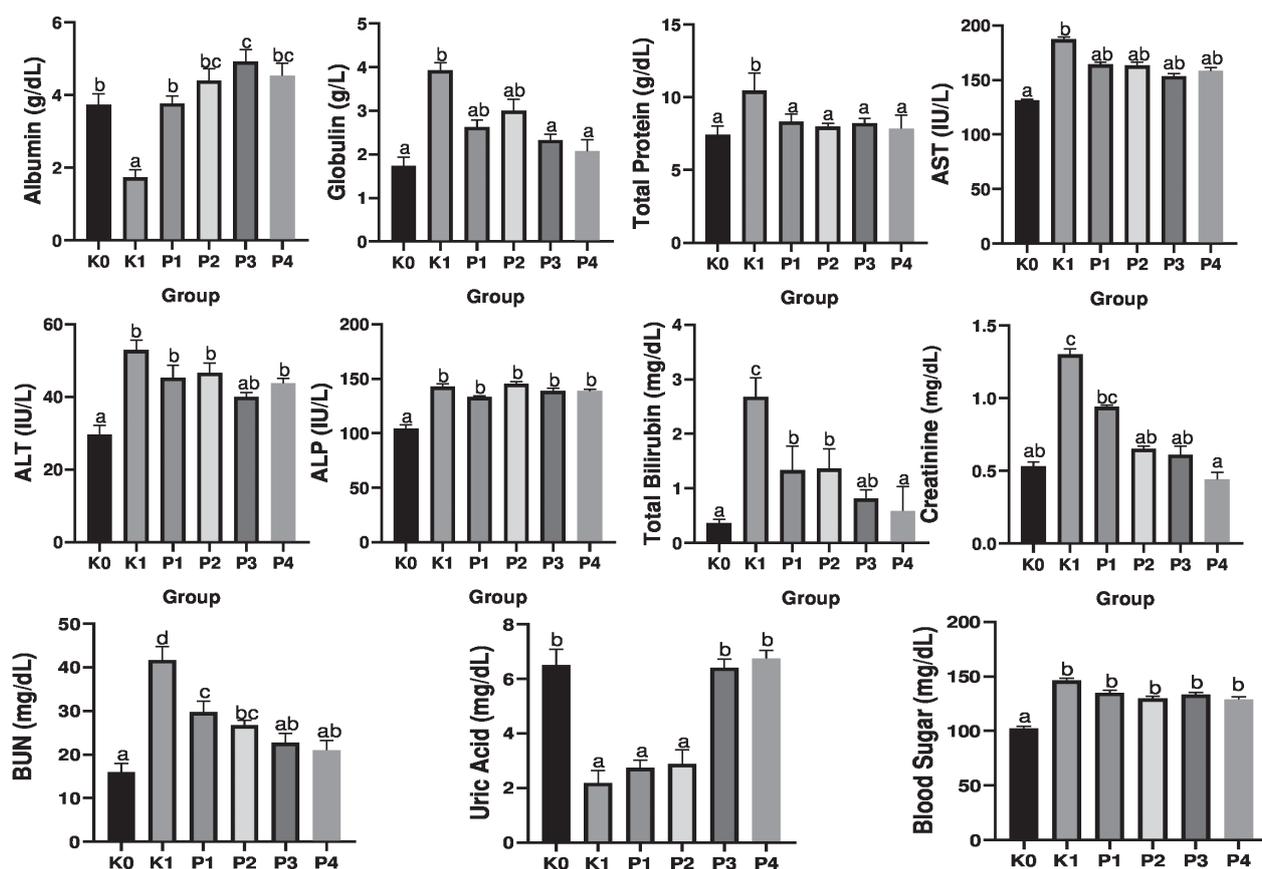


Figure 4. Blood Biochemical Values for Assessing Liver, Kidney, and Pancreas Functions. Panels a, b, c, and d denote statistically significant group differences. K0: Normal, K1: OSCC, P1: OSCC treated with nano herbal *Bischofia javanica*, P2: OSCC treated with nano herbal *Phaleria macrocarpa*, P3: OSCC treated with a combination of nano herbal *Bischofia javanica* and *Phaleria macrocarpa*, P4: OSCC treated with Vitamin C.

Conclusion

The research findings indicate that nano herbals effectively mitigate hematological and lipid damage induced by BaP in OSCC rats. Nano herbals also exhibit the potential to alleviate liver damage, particularly in reducing LDL and total cholesterol levels. This study pro-

vides valuable insights into the potential use of a nano herbal combination of *Bischofia javanica* leaves and *Phaleria macrocarpa* fruits as an innovative approach to cancer treatment. The detailed approach to nano herbal preparation and toxicity testing contributes to understanding the safety and efficacy of nano-based herbal drug applications.

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