OBJECTIVES: Polyphenols in *Phaleria macrocarpa* (mahkota dewa) can inhibit mitogen-activated protein kinase activity in receptor tyrosine kinases pathway. This can be recognized from the decrease of mitotic index as a response to malignant cells and the reduction of tumor development. This study aimed to determine whether *P. macrocarpa* may decrease the mitotic index and tumor diameter in epidermoid carcinoma.

METHODS: The experiment was conducted on 18 epidermoid carcinoma induced Swiss mice divided into four groups: Control, *Phaleria* administered \((0.0715 \text{ mg} \ [0.36 \text{ ml}] / \text{day})\), chemotherapy administered (paclitaxel \(175 \text{ mg/m}^2\) and cisplatin \(50 \text{ mg/m}^2\)), and combination group. Tumor size diameter was measured before and after treatment in 9 weeks. Mitotic index was measured at the end of the treatment.

RESULTS: There were significant differences in the mitotic index and changes in tumor diameter among groups compared with the control group. The most significant growth inhibition and decrease in mitotic index were in group four. There was a significant positive correlation between tumor mitotic index and changes in tumor diameter \((r=0.813)\).

CONCLUSION: *P. macrocarpa* is able to decrease tumor cells’ mitotic index and inhibit epidermoid carcinoma’s tumor mass progression in Swiss mice.

KEYWORDS: *Phaleria macrocarpa*, Mitotic index, Tumor diameter, Epidermoid carcinoma.
week in 2 consecutive weeks. This was followed by the application of 1.7 nmol (0.001 mg) topical 12-o-tetradecanoyl phorbol-13-acetate dissolved in 0.1 ml acetones twice a week in 22 consecutive weeks. The mice were then divided into four groups, consisting of three treatment groups (T1–T3) and one control group (V). Treatment groups in their respective order were treated with (a) 0.0715 mg (0.36 ml/days) of P. macrocarpa extract, (b) 175 mg/m² paclitaxel and 50 mg/m² cisplatin, and (c) 175 mg/m² paclitaxel and 50 mg/m² cisplatin followed by 0.0715 mg (0.36 ml/days) of P. macrocarpa extract in 9 weeks.

Hematoxylin–eosin staining was performed to analyze the mitotic index using the method by Aihara et al. The mitotic cell in 100 tumor cells was counted using ×400, in five different fields of view, then the percentage was calculated. Tumor size was measured using tumor caliper (CalipR) with 1×10⁻² accuracy. The centimeter measurement looked at the longest diameter in every tumor site, taken before and after treatment.

All data were observed as descriptive with mean±SD. The tumor size measurement was performed by using Kruskal–Wallis test. This was followed by Mann–Whitney test to measure the mitotic index in all groups. Spearman test was then used to analyze the correlation between mitotic index and tumor size. All data were analyzed in SPSS version 15 (IBM, USA). The results were considered significant if p≤0.05 and confidence interval was 95%.

RESULTS

After successful tumor induction was confirmed by a pathologist after 9 months, the treatment trials were initiated. After 9 weeks of treatment, the T3 group showed a significant decrease in tumor size compared to pre-treatment (Tables 1 and 2). There was also a significant decrease in mitotic index in all groups (Table 3 and Fig. 1). Normal and homogenous data were obtained from each group in terms of tumor size (p>0.05).

Mann–Whitney test showed that there was a significant difference in tumor size (p=0.049) between V and T1 group, V and T2 group, V and T3 group, and T1 and T3 group (Fig. 2). There was a significant difference in mitotic index between Groups V and T1, V and T2, V and T3, T1 and T3, and T2 and T3 (p=0.043) (Fig. 3).

There was a significant correlation between tumor size and mitotic index (p=0.001) with r=0.813 (Fig. 4). Thus, there was a strong and positive correlation between the decreasing mitotic index and the decreasing tumor size.

Table 1: Mean tumor diameter in each group (in cm)

| Group  | Control | T1  | T2  | T3  |
|--------|---------|-----|-----|-----|
| Before treatment | 1.3 | 0.56 | 1.1 | 0.96 |
| After treatment   | 2.4 | 1   | 1.5 | 0.7  |

Table 2: Average tumor size and mitotic index difference between pre-treatment and treatment group

| Variable          | Mean±SD | Median (min.–max.) | p   |
|-------------------|---------|--------------------|-----|
| Tumor size difference | 0.42±0.537 | 0.4 (–0.5–1.4)   | 0.999|
| Mitotic index      | 1.52±1.046 | 1.1 (0.6–3.4)     | 0.002|

SD: Standard deviation

Table 3: Mitotic index in each group

| Group | Mean±SD | Median (min.–max.) | p   |
|-------|---------|--------------------|-----|
| C     | 3.2±0.346 | 3.4 (2.8–3.4)    | 0.000|
| T1    | 1.13±0.115 | 1.2 (1–1.2)     | 0.000|
| T2    | 1.07±0.115 | 1.0 (1–1.2)     | 0.000|
| T3    | 0.67±0.115 | 0.6 (0.6–0.8)   | 0.000|

Levene test=0.026, C: Control, SD: Standard deviation

DISCUSSION

In this study, we found that there was a significant difference between control group and treatment groups (p=0.049). Even though we did not find any regression in tumor size, a significant inhibition of tumor development was shown in this study. A previous study by Budijitno et al. stated that P. macrocarpa to treat adenocarcinoma mammæ in mice also showed a similar tumor size inhibition effect [15]. Another report demonstrates that P. macrocarpa has a growth-blocking effect in a factor receptor and inhibits MAPK in RTKs signaling pathway [11,12]. Evidence shows how P. macrocarpa blocks several RTKs, such as EGFR, PDGF, and FGR. Thus, it can inhibit RTKs signaling pathway in regulating cell mitotic, differentiation, survival, and cell metabolism by p21 [13,14].

The decrease of mitotic index in treatment groups may have owed to polyphenol contained in P. macrocarpa extract that blocks growth factor receptor in RTKs signaling pathway and inhibits MAPK, causing inhibition in a primary signal transfer of MAPK from the membrane to the nucleus. This condition will push a cell to start the G0 phase and stop mitotic [11,12]. A higher decrease of mitotic index was shown in T3 after the tumor was treated with a combination of chemotherapy drugs paclitaxel – cisplatin and P. macrocarpa at 0.0715 mg/days (0.36 ml/days). The decrease was more significant compared to group treated only with P. macrocarpa extract at 0.0715 mg/days.
CONCLUSION

From this study, we conclude that Phaleria macrocarpa extract has a potent immunomodulator and a cytostatic effect. Clinically, the combination between Phaleria macrocarpa and cytostatic drugs such as Paclitaxel and Cisplatin may reduce the mitotic index of epidermoid carcinoma and may inhibit tumor size. Thus, Phaleria macrocarpa could be used as an alternative adjuvant therapy to cytostatic drugs in treating epidermoid carcinoma.

AUTHORS’ CONTRIBUTIONS

VMS was responsible for study design, data collection, and data analysis. M.TA and D.H were responsible for study design, data analysis, and manuscript writing.

CONFLICTS OF INTEREST

All authors declare that they have no financial conflicts of interest.

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