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CHEMOPREVENTIVE ACTIVITY OF METHANOL EXTRACT OF *MELASTOMA MALABATHRICUM* LEAVES IN DMBA-INDUCED MOUSE SKIN CARCINOGENESIS

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Abstract

Background: *Melastoma malabathricum* L. Smith (family Melastomaceae) is a shrub that has been used by the Malay practitioners of traditional medicine to treat various types of ailments. The present study aimed to determine the chemopreventive activity of methanol extract of *M. malabathricum* leaves (MEMM) using the standard 7,12-dimethylbenz(α)anthracene (DMBA)/croton oil-induced mouse skin carcinogenesis model.
Materials and Methods: In the initiation phase, the mice received a single dose of 100µl/100 µg DMBA (group I-V) or 100µl acetone (group VI) topically on the dorsal shaved skin area followed by the promotion phase involving treatment with the respective test solutions (100 µl of acetone, 10 mg/kg curcumin or MEMM (30, 100 and 300mg/kg)) for 30 min followed by the topical application of tumour promoter (100µl croton oil). Tumors were examined weekly and the experiment lasted for 15 weeks. **Results:** MEMM and curcumin significantly ($p<0.05$) reduced the tumour burden, tumour incidence and tumour volume, which were further supported by the histopathological findings.
Conclusion: MEMM demonstrated chemoprevention possibly via its antioxidant and anti-inflammatory activities, and the action of flavonoids like quercitrin.

Key words: Melastomaceae; skin cancer; anti-carcinogenic activity

Introduction

Cancer is one of the major public health problem areas and remains a major cause of mortality and morbidity in developing as well as in developed countries (Arya, and Kumar, 2011). One of the commercial ways of providing treatments for cancer patients is chemotherapy - using antineoplastic drugs to kill rapid dividing cells. However, these drugs effectiveness have been overshadowed by its associated side effects, which include harm to the rapid dividing cells (i.e. bone marrow, digestive tract and hair follicles) that later lead to immunosuppression, mucositis and hair loss (Nouri, 2008).

Interestingly, the phyto-chemical constituents of some herbs and medicinal plants contain bioactive compounds that displayed potent anti-tumor or anti-carcinogenesis activity and are, therefore, important in the cancer preventive strategy in order to inhibit, delay or reverse carcinogenesis (Surh, 2002). One of the plants that are being studied in our laboratory is a small shrub belonging to the family Melastomaceae known as *Melastoma malabathricum* L. Smith. This medicinal plant has been scientifically reported to possess various pharmacological activities including the antioxidant, cytotoxic and, anti-inflammatory activities (Zakaria *et al*., 2011) that have been associated as parts of the mechanism of anti-carcinogenesis (Arya and Kumar, 2011). It is eminent that there is association between the mechanisms of oxidation inflammation, and cancer and the capability to hamper any of the mechanisms will definitely lead to the inhibition of the others. Nitric oxide (NO), for instance, is generated /liberated under the action of inflammatory stimuli such as the reactive oxygen species (ROS). Hence, inhibition of ROS results in decrease production of NO, which, in turn, triggered the antioxidant, anti-inflammatory, and anticancer processes (Robak and Gryglewski 1988; Middleton et al. 2000; Olszanecki et al. 2002). Interestingly, *M. malabathricum* has been reported to exert antioxidant and anti-inflammatory activities and, therefore, is believed to also possess anticancer activity. Moreover, the free radical scavenging effect is suggested to play important role through which this plant might exert its anticancer activity. However, since these association have not been proven scientifically, the present study was carried out to study the anti-carcinogenesis activity of methanol extract of *M. malabathricum* leaves (MEMM) using the 7,12-dimethylbenz(α)anthracene (DMBA)/crotton oil-induced mouse skin carcinogenesis model.

Materials and Method

Plant leaves collection and preparation of methanol extract

The leaves of *M. malabathricum* were collected around Serdang, Selangor, Malaysia between September and October, 2011 and a voucher specimen (SK 1986/11) was deposited at the herbarium of Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia. The MEMM was prepared according to the method described in detail by Zakaria et al. (Zakaria *et al*., 2011). Basically, from 410 g of dried leaves soaked in methanol (Fischer Scienctific, UK) three times (1:20 (w/v); room temperature for 72 hr) yielded approximately 108.34 g of dried MEMM. The concentrated MEMM was further dried in an oven (40^{o}C) to eliminate excess methanol residue. Prior to use, the MEMM was dissolved in acetone (Mallinckrodt Chemicals, USA) to make up the concentrations of 30, 100 and 300 mg/kg.

Forty eight (48) ICR strain female mice (6-7 weeks old; 20-28 g) were used in this study (Abel *et al*., 2009). The animals were kept in the Animal House, Faculty of Medicine and Health Sciences, UPM and cared according to the standard procedure described elsewhere

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(Zimmermann,1983). An ethical approval was received from the Animal Care and Use Committee of UPM (UPM/FPSK/PADS/BR-UUH/00432). The animals were divided into six groups (n=8) prior to the experimentation.

Three days before the application of DMBA, an area with 2 cm x 2 cm of dorsal skin area of mice was shaved for application of chemicals. Briefly, in the initiation phase, each mouse in group II, III, IV, V and VI received a single dose of 100 µl/100 µg DMBA (Sigma-Aldrich Co, USA) on the dorsal shaved skin area and each animal was held for a few seconds to ensure the chemical distributed evenly on the shaved area before being released back into the cage. On the other hand, all mice in group I only received 100 µl acetone.

Briefly, during the promotion phase, all mice in group I (vehicle control) received only 100 µl of acetone throughout promotion phase. On the other hand, the respective mice in group II (carcinogen control) and III (positive control) received 100 µl of acetone or 10 mg/kg curcumin (Sigma-Aldrich Co, USA) followed 30 min later by the application of 100 µl of croton oil (Sigma-Aldrich Co, USA) throughout the promotion phase. Lastly, the respective mice in group IV, V and VI were treated with 100 µl of 30, 100 and 300 mg/kg MEMM, 30 minutes before the topical application of 100 µl croton oil throughout the promotion phase. All treatments were applied twice weekly for fifteen weeks of promotion period.

Throughout the 15 weeks of experiment, the dorsal skin area was observed carefully for any papilloma growth as described in detail by (Abel *et al*., 2009). Briefly, several important parameters such as body weight, latency period of tumor formation, percentage of tumor incidence, tumor burden and tumor volume were also recorded weekly. The experiment was terminated on the 15th week, and all animals were sacrificed by using diethyl ether and the dorsal skin was shaved and dissected out for histo-pathological analysis. The dissected skin tissues then underwent the standard Haematoxylin and Eosin (H&E) staining procedure as described by Abel *et al.*

All data are presented as Mean ± Standard Error of Mean (S.E.M.). The data were statistically analyzed using the one-way analysis of variance (ANOVA) followed by LSD multiple comparison test. Values with $p \leq 0.05$ were considered as statistically significant.

Results

The body weight of mice receiving acetone, 10 mg/kg curcumin or, 30 and 100 mg/kg MEMM increased gradually until week 15th (data not shown). The initial and final body weight of mice in Group I to VI is shown in Table 1. Comparison between the initial (before promotion) and final body weight (15th week after promotion) demonstrated significant ($p<0.05$) increased in body weight in all groups except for the group receiving 300 mg/kg MEMM. This finding was supported by data for percentage of tumor incidence obtained at the end of experimental weeks (15th week) wherein group II showed the highest tumor incidence (75.0%) in comparison with Group I, III, IV, V and

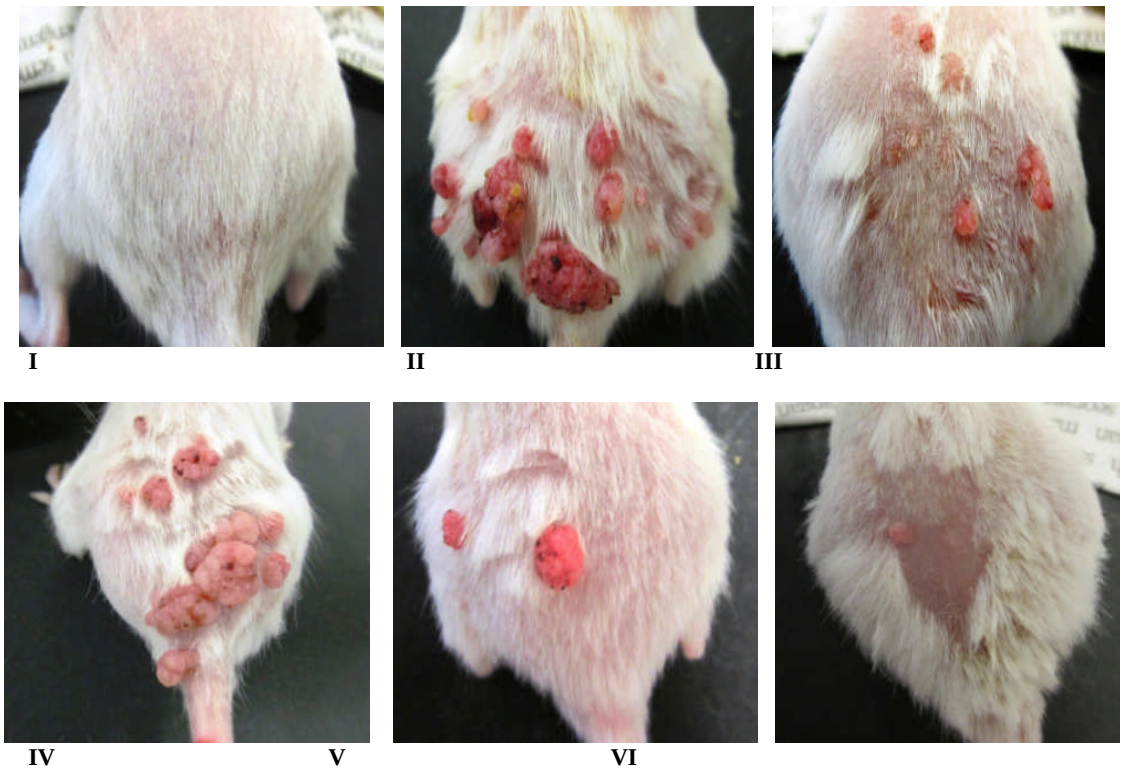

Figure 1. The gross appearance of the skin papilloma on the shaved dorsal skin of DMBA/croton oil-treated mice following the topical administration of MEMM or curcumin on week 15th. Representative photographs for each experimental group captured at the end of study. (I) Group I: vehicle control only applied with acetone; (II) Group II: carcinogen control only applied with 1% croton oil at the promotion stage and without any pre-treatment; (III) Group III: positive control treated with 10 mg/kg body weight curcumin; (IV) Group IV: treated with 30 mg/kg body weight MEDL; (V) Group V: treated with 100 mg/kg body weight MEDL, and; (VI) Group VI: treated with 300 mg/kg body weight MEDL.

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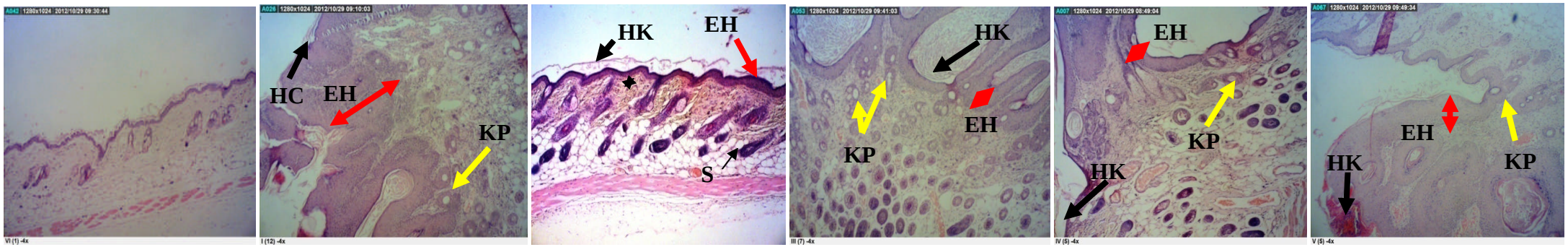

Figure 2:. Microphotographs of skin tissue from different groups after pretreatment with acetone, croton oil, curcumin or MEMM. (40x magnification). I) Microphotographs of skin tissue from acetone (vehicle control) showed well-defined histology of skin with intact basement membrane. The basement membrane is well defined, with no signs of invasions into dermis layer.; II) Microphotographs of skin tissue from pre-treatment with 1% croton oil (carcinogen control) showed the keratin pearls (KP) found within the papilloma (yellow arrow) with mild hyperchromatism (HC) (black arrow) surrounding the pearls. III) Microphotographs of skin tissue from pre-treatment with 10 mg/kg curcumin (positive control) showing the papilloma and hyperplasia (red arrow) with no disruption of basement membrane (black arrow); IV) Microphotographs of skin tissue from pre-treatment with 30 mg/kg MEMM showing a clear structure of papilloma and hyperplasia of epidermis (EH) (red arrow) with defined basement membrane; V) Microphotographs of skin tissue from pre-treatment with 100 mg/kg MEMM showing the papilloma was protruding from the epidermis (red arrow) with hyperplastic changes of epidermis (black arrow), the structure inside the papilloma are hair follicle (hf); and VI) Microphotographs of skin tissue from pre-treatment with 300 mg/kg MEMM showing the papilloma has growth outwards the skin with hyperplasia on epidermis. Abbreviation: HK - Hyperchromatins ; EH – Epidermis hyperplasia; KP – Keratin pearls; S -

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VI (tumor incidence range between 0-62.5%) (Table. 1). There are significant ($p < 0.05$) increased in tumor incidence with increase in period of exposure to carcinogenic agent, which were significantly ($p<0.05$) reduced following pre-treatment with curcumin or MEMM (100 and 300 mg/kg). Groups receiving curcumin and MEMM exerted significant ($p<0.05$) reduction in tumor burden and tumor volume at the end of 15th week with the latter occurred in a dose-dependent manner (Table 1). The tumor burden increased from Group I to Group VI followed by Group III, V, IV and II. The largest tumor volume was found in Group VI followed by Group V, IV, II and III. Group I has no tumor volume. As for the latency period of tumor formation, curcumin and MEMM (30, 100 and 300 mg/kg) delayed the formation of tumor until week 10th, 8th, 9th and 14th, respectively in comparison to the negative control group (week 7th) (data not shown). The overall gross appearance of the shaved dorsal skin of a representative mouse from all the treatment groups on week 15 prior to dissection supported the ability of curcumin and MEMM to exert anti-carcinogenic activity (Figure 1) and supported by the microscopic examination (Figure 2).

Discussion

Results obtained from the present study confirmed the novel anti-carcinogenic activity of MEMM in DMBA/croton oil-induced mice. The DMBA initiates skin tumor formation through the generation ROS that are responsible for genetic materials damage (Das *et al.*, 2010). Therefore, the chemoprevention activity of MEMM could be attributed to the extract's antioxidant activity, which, in turn, could be associated with the extract's phytochemical constituents (e.g. flavonoids, triterpenes, tannins and saponins) (Surh, 2002; Zakaria *et al*., 2011). MEMM has demonstrated high antioxidant and free radical scavenging potential when assessed by the DPPH radical scavenging and superoxide scavenging assays suggesting these effects direct or indirect contribution to the extract's observed anti-carcinogenic activity (Li et al. 2008). Compounds like flavonoids (Ferguson et al. 2004), saponins (Lemeshko et al. 2006), triterpenes (Kimura et al. 2002) and tannins (Ait Mbarek et al. 2007) have all been reported to possess antioxidant and anticancer activities and are, therefore, believed to act synergistically within the respective extract to cause the observed anti-carcinogenic activity.

In term of the mechanisms of anti-carcinogenesis that might be modulated by MEMM, several pathways could be suggested based on the flavonoids detected in MEMM. Flavonoids are important in the impediment of various chronic maladies involving oxidative stress and demonstrated antioxidant, anti-inflammatory and anticancer activities via the in vivo and in vitro models (Lee et al. 2003; Middleton et al. 2000; Robak and Gryglewski, 1988; Calixto et al. 2003; 2004). Moreover, flavonoids also regulated the expression of pro-inflammatory genes (i.e. nitric oxide synthase (NOS) and cyclooxygenase-2 (COX-2)) (Dawson and Snyder, 1994; Kim et al., 2004).

Interestingly, we have also detected quercitrin in the MEMM (Mamat et al., 2013), which have been earlier reported to exert in vitro anti-carcinogenic activity against mouse JB6 cells and antioxidant activities (Ding et al., 2010). Moreover, quercitrin down-regulates transactivation of AP-1 and NF-κB induced by UVB or TPA. Quercitrin was also shown to inhibit several biochemical pathways (i.e. ERKs, p38 kinase, and JNKs) related to cancer via the inhibition of MAPKs phosphorylation. In addition, quercitrin stimulated the activation of NF-E2-related factor (Nrf2) and GST ARE-luciferase activity.

On the other hand, curcumin had been chosen as the positive control in comparison to the MEMM since curcumin contains anti-inflammatory properties which lead to its effectiveness as a chemo-preventive agent (Sonavane et al., 2012). In conclusion, MEMM exhibited chemoprevention activity, which is attributed, partly, to its antioxidant activity and phyto-constituents, particularly, quercitrin.

Conflict of interest: The authors declare that there is no conflict of interest.

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