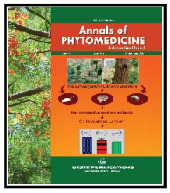
Annals of Phytomedicine 11(1): 276-282, 2022

DOI: http://dx.doi.org/10.54085/ap.2022.11.1.28

Annals of Phytomedicine: An International Journal

http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Platelet boosting effect of commercial herbal supplements in chemically-induced thrombocytopenic rats

Sheri-Ann Tan*, Akash Parthi Bendran*,*, Mohammad Razak Hamdan*, Nur Azzalia Kamaruzaman, Suresh Rajoo***, Tarun Agarwal****, Surash Ramanathan* and Vikneswaran Murugaiyah*,*****,◆**

Department of Bioscience, Faculty of Applied Sciences, Tunku Abdul Rahman University College, 53300, Kuala Lumpur, Malaysia

**Centre for Drug Research (CDR), Universiti Sains Malaysia, 11800 Penang, Malaysia*

***National Poison Centre, Universiti Sains Malaysia, 11800 Penang, Malaysia*

****School of Healthy Aging, Medical Aesthetic and Regenerative Medicine, UCSI University, 56000 Kuala Lumpur, Malaysia*

*****Department of Bio-Technology, Koneru Lakshmaiah Education Foundation, Vaddeswaram, AP, India*

******Discipline of Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia*

Article Info

Article history

Received 21 April 2022

Revised 11 June 2022

Accepted 12 June 2022

Published Online 30 June 2022

Keywords

Antioxidant

Anti-thrombocytopenia

Platelet

Spices

Herbs

Phenolics

Abstract

Thrombocytopenia is a hematologic disease that leads to low platelet levels in patients. This disorder is multifactorial and could be due to ailments such as cancer and viral infections like dengue. Commercially available phenolic-rich herbal supplements (Manna Plus and Semalu) containing medicinal herbs were found to assist in restoring normal platelet count in dengue-affected patients. Therefore, the main aim of this research is to investigate the platelet boosting properties of both these supplements by employing a cyclophosphamide-induced thrombocytopenic rat model system. The phenolic and flavonoid contents along with the antioxidant activities of the ethanolic extracts from Semalu (SEE) and Manna Plus (MEE) supplements were determined as well. Results revealed that the ethanolic extracts from both supplements possessed antioxidant activity as well as platelet boosting properties. The platelet count in the chemically-induced thrombocytopenic rat models subjected to the ethanolic extracts reached normal levels while the negative control group did not show a significant recovery. Interestingly, the platelet recovery in thrombocytopenic rats treated with the ethanolic extracts was achieved at 100-folds lower than the dose applied for papaya leaves extract (positive control group). In conclusion, the current data suggests that these edible ayurvedic spice supplements may be useful in treating thrombocytopenic diseases, including chemically-induced thrombocytopenia, which can often occur in cancer therapy.

1. Introduction

Thrombocytopenia is a blood disorder that causes drastic reduction in platelet count (Izak *et al*., 2014) due to (i) decreased in platelet generation, (ii) enhanced platelet destruction and (iii) altered distribution of platelets (Manasa *et al*., 2016). This condition is often associated with liver disease (Afdhal *et al*., 2008), cancer chemotherapy (Liebman *et al*., 2014) and viral infections such as dengue and Covid-19 (Bhattacharjee and Banerjee, 2020). The traditional treatment for thrombocytopenia includes initial prescription of corticosteroids and if this fails, platelet transfusion or splenectomy is required. Recently, thrombopoietin receptor agonists and spleen tyrosinase kinase inhibitors are also developed to treat this hematologic condition (Samson *et al*., 2019). However, all these therapies do come with side effects and remission rate remains low.

Literature had also reported many platelet-boosting herbal plants that could facilitate the recovery process of dengue patients. For instance, the asthma weed plant, *Euphorbia hirta*, is used by the indigenous communities in the Philippines for dengue treatment (Manasa *et al*., 2016). Another popular plant remedy for this viral infection is *Carica papaya*, a tropical plant. Several studies had reported the potency of papaya leaf extracts in boosting platelets (Subenthiran *et al*., 2013; Patil *et al*., 2013; Anjum *et al*., 2017). Interestingly, all these plant extracts also exerted antioxidant potentials. In fact, there is a clear association between oxidative stress and thrombocytopenia reported in dengue infection where severe lipid peroxidation was observed leading to platelet destruction (Soundravally *et al*., 2008). Therefore, antioxidants could indeed improve the outcome of this hematologic disorder.

In Malaysia, there are numerous claims that the herbal products, Semalu and Manna Plus, produced by Biospektra could enhance platelet levels in dengue patients, hence, alleviating the severity of this infection. Semalu is a mixture containing *Azadirachta indica* A. Juss. (neem), *Curcuma longa* L. (turmeric), *Allium cepa* L. (onion) and *Allium sativium* L. (garlic). Manna Plus, comprises onion, garlic, *Nigella sativa* L. (Black cumin), *Cuminum cyminum* L. (cumin seeds), *Zingiber officinale* Roscoe (ginger), *Syzygium aromaticum* L. (clove), *Coffea arabica* L. (coffee) and *Anethum graveolens* L. (dill). Semalu is claimed to prevent urinary tract infection (UTI), increases vaginal strength as well as a complementary treatment for pimples or even severe acne cases (Biospektra, 2021a). As for Manna Plus, it is said

Corresponding author: Dr. Vikneswaran Murugaiyah

Centre for Drug Research (CDR), Universiti Sains Malaysia, 11800 Penang, Malaysia

Discipline of Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

E-mail: vicky@usm.my

Tel.: +604-6574149

to regulate the physiological balance of the body and indirectly improving the number of blood cells (Biospektra, 2021b). Both these products are initially produced for general health benefits and contained ayurvedic herbs as their main ingredients. Since both these supplements are now perpetually being sold as an adjunct treatment for dengue rather than for well-being purposes, more research ought to be conducted on these supplements especially in their platelet enhancing properties, particularly in thrombocytopenia caused by other conditions besides dengue.

In the present work, a methodological demonstration of the antioxidative strength of the ethanolic extracts of Semalu and Manna Plus was done before testing their platelet boosting potentials using cyclophosphamide-induced thrombocytopenia murine model. Cyclophosphamide, an alkylating agent, is often used in cancer chemotherapy and is associated with platelet destruction through oxidative stress by overloading the spleen and liver with iron besides downregulating Nrf-2 gene expression (Sheng *et al*., 2020). Notably, treatment with antioxidants was shown to reverse this mechanism (Wang *et al*., 2021). Hence, we considered this model system for our investigation.

2. Materials and Methods

2.1 Materials

For the HPTLC analysis, chemical standards used were: eugenol, curcumin, linolenic acid and quercetin from Acros Organics. Other chemicals and reagents especially for antioxidant assays were purchased from Merck and Sigma Aldrich. HPLC grade methanol and potassium dihydrogen orthophosphate were also used. All other chemicals were of AR grade. The herbal supplements; Semalu and Manna Plus were obtained from Dimesoft Sdn. Bhd.

2.2 Animal maintenance

Male Sprague-Dawley rats (12 to 16 weeks old) weighing 250–300 g, were obtained from Universiti Sains, Malaysia. The animals were kept in a room at a constant temperature of 25°C with a 12-h light/ dark cycle. Rats were allowed free access to water and standard food pellets (Gold Coin, Malaysia). This study was approved by the Animal Ethics Committee of Universiti Sains Malaysia, Penang [USM/2015/ (678)]. Institutional guidelines were adhered when handling the animals before and during the commencement of experiment. Twelve (12) hours before the start of experiment, food was withdrawn but the rats had free access to water. Tail nick blood sampling method (Abatan *et al*., 2008) was used in this study to sample blood from test animals.

2.3 Preparation of SEE and MEE

Powdered materials were removed from the capsules of Semalu and Manna Plus. The contents were macerated repeatedly in 96% ethanol (v/v) for three days. The extracts obtained were filtered and concentrated using a rotary evaporator (IKA, Germany). Concentrated extracts were frozen dried and the yield of extraction was determined. Final product was stored in a 4°C refrigerator until further analysis.

2.4 Characterization of phytochemical contents and antioxidant activities of SEE and MEE

An estimation of free radicals scavenging activity was quantified using DPPH assay as described (Shimada *et al*., 1992). Ferric ion reducing antioxidant power was obtained by the FRAP method (Benzie and Strain, 1996). Results from both assays were expressed as trolox equivalent (TE)/ g dry weight of extract. The total phenolic contents of the extracts were determined based on the reduction of phosphomolybdic-phosphotungstic acid (Folin) reagent to a blue-colored complex in the presence of phenolic compound. The intensity of light absorption at wavelength 765 nm was proportional to the concentration of phenols and results were expressed in gallic acid equivalent (GAE)/g dry weight extract (Kaur and Kapoor, 2002).

2.5 Evaluation of platelet boosting properties of SEE and MEE in thrombocytopenic rat model

Rats were randomly assigned into six experimental groups (n = 6, each). Blood was drawn one day prior to the onset of the experiment which served as baseline blood samples. Control animals received 20% Tween 20 solution (5 ml/kg, p.o) for five consecutive days. The negative control animals were given cyclophosphamide (CY) (50 mg/kg x 1 per day, i.p) for three consecutive days (Patil *et al*., 2013). The positive control animals received the CY (50 mg/kg x 1 per day, i.p) for three consecutive days, followed by papaya leaf extract (500 mg/kg x 3 per day, p.o) for three days in a row (Subenthiran *et al*., 2013). For the test animals, toxicant CY (50 mg/kg x 1 per day, i.p) was given for three consecutive days and after that the rats were subjected to (i) Semalu extract (5 mg/kg x 3 per day, p.o), (ii) Manna Plus extract (5 mg/kg x 3 per day, p.o) and (iii) a combination of Semalu and Manna Plus Extracts (5 mg/kg each x 3 per day, p.o) for three consecutive days, respectively. For all treated animals, blood was drawn at day three and day five as illustrated below.

	Blood Drawn ↓		Blood Drawn ↓		Blood Drawn ↓
GROUPS	**DAY 1**	**DAY 2**	**DAY 3**	**DAY 4**	**DAY 5**
Control	Tween 20	Tween 20	Tween 20	Tween 20	Tween 20
Negative Control	**CY**	**CY**	**CY**	-	-
Positive Control	**CY**	**CY**	**CY** PLE	PLE	PLE
Test Groups	**CY**	**CY**	**CY** Test Sample	Test Sample	Test Sample

CY: cyclophosphamide, **PLE:** papaya leaf extract

2.6 Analysis of chemical constituents in SEE and MEE

Linear ascending development was executed in a 10 × 10 cm^2 twin trough glass chamber (CAMAG, Switzerland) using a mobile phase of chloroform, methanol and formic acid at ratio of 8:2:1 (v/v/v). Merck HPTLC plates (Silica Gel 60 F254, 10 × 20 cm) were used as stationary phase. Samples were applied using CAMAG Linomate V semiautomatic sample applicator. Identification and quantification of phytocompounds (eugenol, curcumin, linoleic acid, quercetin and caffeine) of the spice mixtures were performed by TLC densitometry using CAMAG TLC Scanner 3 and WinCATS software version 1.3.4.

2.7 Statistics

All data were expressed as means ± S.E.M. Unpaired t-test was used to compare two sample groups for TPC, DPPH and FRAP assays. The effects of various extracts on their platelet boosting abilities were analyzed using one-way ANOVA, followed by Bonferroni post-

hoc test. Probability values less than 5% ($p < 0.05$) were considered significant. Statistical analysis was performed using SPSS version 26.0.

3. Results

3.1 Antioxidative potential of ethanolic extracts of Semalu and Manna Plus

The antioxidant capacities of these supplements were tested and both SEE as well as MEE showed free radical scavenging properties. SEE (347.03 mg Tr/g extract) was revealed as a better radical scavenger when compared to MEE (317.17 mg Tr/g extract). This coincided with the higher amount of phenolic found in SEE (35.83 mg GAE/g extract) when compared to MEE (18.85 mg GAE/g extract) as illustrated in Table 1. Interestingly, in the FRAP assay, MEE showed higher reducing potential (1.67 Tr/g extract) than SEE (1.38 g Tr/g extract) despite the former possessing phenolic content of approximately 2-fold lower than SEE (Table 1).

Table 1: Total phenolic content, DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP) of Semalu ethanolic extract (SEE) and Manna Plus ethanolic extract (MEE)

Extracts	Total phenolic content (TPC) (mg GAE/g extract)	DPPH (mg Tr/g extract)	FRAP (g Tr/g extract)
SEE	35.83 ± 3.06^a	347.03 ± 22.24^a	1.38 ± 0.09^a
MEE	18.85 ± 1.68^b	317.17 ± 7.03^b	1.67 ± 0.03^b

Note: GAE: Gallic Acid Equivalent, **Tr**: Trolox Equivalent. Standard curve (R^2) for DPPH and FRAP = 0.99. Values are presented as mean ± S.E.M. (n = 3). Means with different superscript letters are significantly different ($p < 0.05$) when comparing Semalu and Manna Plus for each assay using unpaired sample t-test.

3.2 Anti-thrombocytopenia effects of Semalu and Manna Plus ethanolic extracts

Prior to testing the extracts on the thrombocytopenia rat model, ethanolic extracts of both supplements were first tested on healthy rats. The dosages of the extracts were calculated based on the prescribed doses stated on the labels of the products. Each capsule of Semalu and Manna Plus contained 250 mg ingredient of the dry powder. Recommended daily dose for general health was 2 capsules per day. Taking an average of 60 kg human body weight, the dose consumed by human was around 8.33 mg/kg. For application in rats, animal equivalent dose was considered (Reagan-Shaw *et al*., 2008). Since ethanol extract was used instead of the powder, 10% extract yield was taken into consideration for total final dose administration. The calculated total doses of the SEE and MEE were both 5 mg/kg/day, respectively. This was given to the rats as 1.7 mg/kg x 3 times per day.

The platelet count of control animals receiving only Tween 20 solution (20%) on the initial day, day 3 and day 5 were 909, 831 and 845 x 10^9/l, respectively. The corresponding platelet counts for SEE and MEE treated groups (1.7 mg/kg x 3 times per day) were 933, 849 and 900 x 10^9/l and 959, 823 and 919 x 10^9/l, respectively. From our observation, the platelet levels in healthy rats upon treatment with either extract fell within the normal range of healthy control rats (Figure 1).

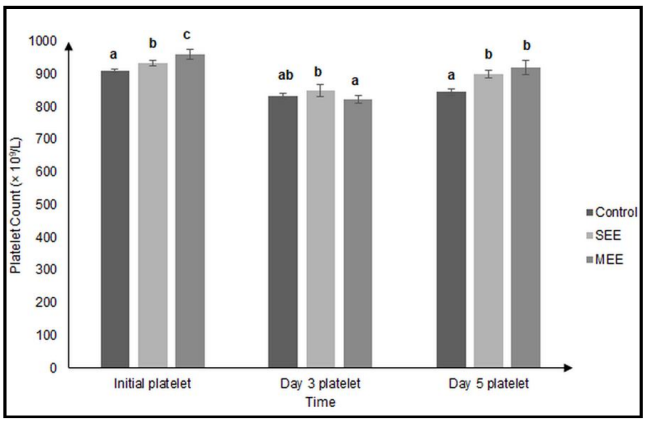


Figure 1: Platelet count in rats after administration of SEE (1.7 mg/kg x 3 times per day x 3 days) and MEE (1.7 mg/kg x 3 times per day x 3 days) over a five-day duration period. Values are presented as mean ± S.E.M. (n = 3) and were statistically evaluated using one-way ANOVA and Bonferroni post-hoc tests. Different alphabets within each time frame indicate significant difference between the tested samples at $p<0.05$.

Rats were induced with thrombocytopenia utilizing the model system proposed by Mivechi and Ogilvie (1989) with appropriate cyclophosphamide (CY) dose modification (Patil *et al*., 2013). The CY at 50 mg/kg x 1 time per day for 3 days conferred a suboptimal reduction of platelet count in murine model (Bendran, 2016). Determination of SEE and MEE doses for platelet boosting abilities in thrombocytopenia rats was based on similar approach used for SEE and MEE dosage calculations to boost platelet count in healthy rats. The recommended dose for dengue patients was two capsules of Semalu and Manna Plus thrice a day. Hence, the estimated doses for both SEE and MEE were 5 mg/kg x 3 times per day for three consecutive days, respectively. This dose was used to study the platelet boosting properties of SEE and MEE along with their combinations in thrombocytopenic rats. In control animals having received only the vehicle (Tween-20), the platelet counts did not drop significantly over time (Initial day: 909 x 10^9/l; Day 3: 831 x 10^9/l; Day 5: 844 x 10^9/l). Conversely, for the negative control group where only CY was administered (50 mg/kg x 1 time per day x 3 days), a suboptimal decline in platelet count (Initial day: 854 x 10^9/l; Day 3: 406 x 10^9/l; Day 5: 300 x 10^9/l) was observed (Figure 2).

The remaining test groups treated with CY (50 mg/kg x 1 time per day x 3 days) were then challenged with SEE, MEE and papaya extract (positive control group). The thrombocytopenia animals (Day 3: 453 x 10^9/l) challenged with papaya extract resulted in platelets counts returning to normal levels (Day 5: 784 x 10^9/l). In test animals (thrombocytopenia rats) challenged with SEE the platelet count went up to 729 x 10^9/l on Day 5 from 438 x 10^9/l on Day 3, which was comparable to the positive control (papaya extract group). A corresponding increment of platelet count was also encountered in MEE test group (Day 3: 468 x 10^9/l; Day 5: 714 x 10^9/l). The combination effect of both SEE and MEE was tested at the dose of 5 mg/kg and the platelet boosting effect was found to be significantly similar to that of the extracts when they were given alone (Day 3: 446 x 10^9/l; Day 5: 720 x 10^9/l). The platelet level eventually rose to a normal range which was comparable to positive control. Overall observation showed that the platelet count increase to nearly normal

range (700 to 900 x 10^9/l) in CY-induced thrombocytopenia animals given either the individual extracts or in combination (Figure 2).

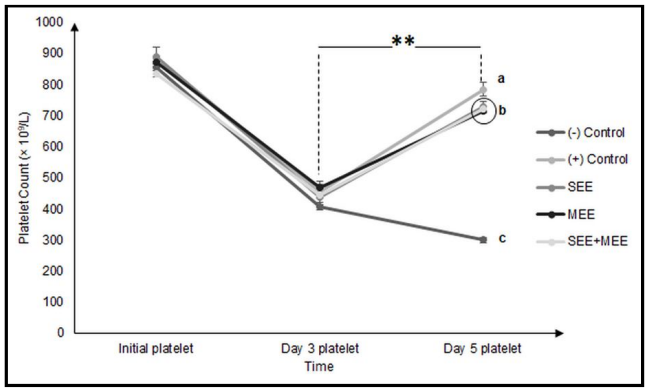


Figure 2: Platelet count in rats given the cyclophosphamide 50 mg/kg x 1 time per day x 3 days (Negative Control) challenged with papaya extract (Positive Control), SEE MEE and combination of both supplements. Values are presented as mean ± S.E.M. (n=3) and were statistically evaluated using one-way ANOVA and Bonferroni post-hoc tests. Note: The symbol ** represents significant difference from initial day whereby different alphabets (a, b and c) indicate significant difference between tested groups on Day 5.

3.3 Chemical standardization of Semalu and Manna Plus ethanolic extracts

SEE and MEE were eventually standardized using four different phytocompounds. Eugenol and curcumin were detected in SEE and MEE as illustrated in Table 2. However, linoleic acid and quercetin were found only present in MEE. Caffeine, on the contrary, was not identified in both ethanolic extracts.

Table 2: Standardization of Semalu ethanolic extract (SEE) and Manna Plus ethanolic extract (MEE) based on the presence of eugenol, curcumin, linoleic acid and quercetin.

Amount of standards present in the extracts (µg/mg extract)		
Standards	**MEE**	**SEE**
Eugenol	0.60	0.40
Curcumin	0.24	0.48
Linoleic acid	0.28	-
Quecertin	0.36	-

Note: Amount of standards in 1 mg of the extracts expressed in µg/mg.

4. Discussion

Cold maceration technique was used in the extraction of phytochemicals from Semalu and Manna Plus supplements to conserve thermo labile compounds during the process. High temperatures were avoided as it may cause degradation of certain compounds, and thus reduced the quality of the extracts (Jones and Kinghorn, 2012). Ethanol, a solvent of high polarity, was employed in the maceration process as it would enhance the extraction efficiency of polyphenols that were often associated with pharmacological properties (Agarwal *et al.*, 2021).

In order to be characterized as an antioxidant, MEE and SEE should be able to effectively scavenge reactive oxygen species (ROS), prevent radical chain reaction or eliminate production of free radicals (Huang *et al.*, 2005). The most common method to screen plant extracts for their antioxidant activity is the DPPH assay. DPPH is a free radical that is readily neutralized into a stable diamagnetic molecule by accepting a hydrogen radical or electron (Kroyer, 2004). Once the reaction is completed, the intense purple of DPPH transforms into pale yellow indicating free radical scavenging activity (Blois, 1958). Both the SEE and MEE possessed free radical scavenging properties with SEE (347.03 mg Tr/g extract) exerting slightly better response than MEE (317.17 mg Tr/g extract). This coincided with the higher amount of phenolic compounds found in the case of SEE (35.83 mg GAE/g extract) when compared to MEE (18.85 mg GAE/g extract) as depicted in Table 1. Numerous reports had mentioned a positive correlation between antioxidant activities of plant extracts to their phenolic contents (Sethumathi *et al.*, 2021; Gadade *et al.*, 2019; Qader *et al.*, 2011; Prasad *et al.*, 2009; Yen *et al.*, 1993). The phenolic and flavonoid compounds present in both of these herbal supplements could be the principal constituents contributing to their antioxidant activities (Malik *et al.*, 2020).

Nevertheless, in the FRAP assay, similar trend between antioxidant activities and phenolic contents was not observed. MEE showed slightly higher reducing potential (1.67 g Tr/g extract) than SEE (1.38 g Tr/g extract) despite the former possessing phenolic content approximately 2-fold lower than SEE. The reducing ability of an extract in this assay was measured based on the conversion rate of ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to a blue-colored product of ferrous tripyridyltriazine (Fe^{2+}-TPTZ) which depended on the presence of reductones in the extracts (Benzie and Strain, 1996). The difference in antioxidant activities between these two extracts in both assays (DPPH and FRAP) could be attributed to the stereo-selectivity of radicals in these two testing systems, hence, indirectly affected the quenching capacity of the bioactive compounds (Jabri Karoui and Marzouk, 2013). It is important to note that, no single assay determination could precisely reflect the total antioxidant capacity of these products as this involved radical sources or antioxidants in a mixed or complex system (Gezici *et al.*, 2020). SEE and MEE were then proceeded to be tested for their platelet boosting abilities

Before SEE and MEE were tested on thrombocytopenic rats, the extracts were experimented initially on healthy animals, where it was found that the extracts maintained the platelet levels within the normal range of healthy control rats. Based on literature, normal healthy rats charted platelet count ranging from 700 to 900 x 10^9/l (Joris *et al.*, 1980; Patil *et al.*, 2013). Therefore, this clearly suggested that the products at the recommended doses did not exhibit thrombocytosis effects or platelet stimulating properties in healthy rats (Figure 1).

In order to induce thrombocytopenia in the rat model, an immunosuppressant drug, cyclophosphamide (CY), was administered. High dosage of cyclophosphamide could drastically affect the animal's homeostasis and thus led to complications which might not be relevant to platelet decrease. Thus, CY at a suboptimal dose would be best used in this study. Given the lack of data supporting the suboptimal dose required to induce thrombocytopenia, we prospectively studied the CY dose response to platelet reduction

in rats. We found CY at 50 mg/kg x 1 per day x 3 days conferred a significant reduction of platelet count in murine model (Bendran, 2016). The animals having treated with the CY had a significantly lower platelet count at day 3 and 5 when compared to control animals. Thrombocytopenic rats given papaya extracts (positive control) showed significant improvement by returning platelet counts to normal range. This data was in line with the observation reported by other research groups (Anjum *et al*., 2017; Patil *et al*., 2013). Importantly, SEE and MEE also demonstrated increment of platelet levels back to the normal range despite the dosages of both these extracts (5 mg/kg) was 100-folds lower than the papaya leaves extract (500 mg/kg) (Figure 2). Results obtained may indicate higher potency of SEE and MEE extracts in comparison to papaya leaves extract in platelet boosting but this warranted further investigation.

Previously, papaya leaves extract, the positive control in this study, was investigated by Anjum and colleague (2017) for its antithrombocytopenic effect using the same murine model system. Treatment with the standardized aqueous extract prominently elevated the platelet count in cyclophosphamide-treated rats. The extract also exhibited high antioxidant as well as immunomodulatory properties which could contribute to the reversal of the adverse effects observed.

In view of that, we postulated that the antioxidative potential of Semalu and Manna Plus could alleviate the oxidative stress effects in the murine model after treatment with cyclophosphamide and therefore, improved the platelet count as observed on Day 5. Besides papaya leaves, the phytochemical, bergenin, isolated from the plant genus *Bergenia*, showed similar properties as the herbal supplements when given to cyclophosphamide-immunosuppressed mice by preventing the drop in platelet count (Qi *et al*., 2018). Additional work done by this research group concluded that the alleviation of adverse effects conferred by cyclophosphamide through bergenin treatment was mainly due to its antioxidative strength and immunomodulatory activities. In fact, many plant extracts and even isolated phytochemicals are currently used as adjuvant therapy for thrombocytopenia especially in dengue cases (Ahmad *et al*., 2021; Navak *et al*., 2019; Manasa*et al*., 2016).

Inconsistency of bioactivities in similar herbal plants or different plant parts within the same plant could be influenced by the geographic location, soil fertility and also variation in handling during harvesting of raw materials. All these factors directly affect the production of phytochemicals in a particular plant. Furthermore, these herbal products are liable to deterioration over time and are exposed to contamination from other substances (Ekor, 2014). Due to the complex and varying composition of plant chemicals, the World Health Organization (WHO) emphasized the importance of ensuring the quality of herbal plant products with medicinal values by means of modern standardization techniques (WHO, 2011). Therefore, methodologies that promote the development of chemical fingerprints of crude herbal extracts are convenient standard protocols to detect the stability of extracts over time (Agarwal *et al*., 2021).

SEE and MEE were chemically standardized to ensure that extracts of similar qualities were utilized throughout this study. Phyto-constituents used in the standardization procedure were curcumin, eugenol, linoleic acid and quercetin. Both extracts showed the presence of curcumin and eugenol, while MEE revealed the existence of additional linoleic acid and quercetin. The high eugenol content in MEE (0.6 µg/mg extract) could be contributed by clove, one of the ingredients in Manna Plus (Biospektra, 2021b). Cucurmin, on the other hand, was found at a higher quantity in SEE (0.48 µg/mg extract) than MEE (0.24 µg/mg extract) due to the presence of turmeric in Semalu (Biospektra, 2021a). Nevertheless, there were no traces of caffeine in both extracts (Table 2).

5. Conclusion

Semalu and Manna Plus herbal products mainly consist of edible ayurvedic spices and have been long marketed as general health supplements in Malaysia. In this study, both phenolic-rich SEE and MEE demonstrated strong platelet boosting abilities in our thrombocytopenia rat model system. In addition, antioxidative activities of SEE and MEE were observed. The presence of eugenol, curcumin, linoleic acid and quercetin in Manna Plus were also detected and quantified, while eugenol and cucurmin were found in Semalu. These phytochemicals could be used as biomarkers to standardize the preparation of these herbal extracts for future work. Oral administration of SEE and MEE was able to recover blood platelets back to the normal physiological range in the CY-induced thrombocytopenic rat model. It is of importance to note that the underlying mechanistic actions of these products in platelet boosting observed in thrombocytopenia rat model and dengue patients remain to be elucidated and warrant for detailed investigation. At this juncture, our present findings experimentally supported that these products may be used as health supplements to boost thrombopoiesis when platelet counts are impaired by diseases. With regard to toxicity, no adverse reactions have been reported or published on these products. In short, results from this study indicated that the standardized phenolic-rich supplements demonstrated promising anti-thrombocytopenia effects on the rat model system besides possessing antioxidative strength.

Acknowledgements

The authors wish to acknowledge the financial support given by Universiti Sains Malaysia (USM) for this research study.

Equal contribution

* **: Sheri-Ann Tan and Akash Parthi Bendran**

Author contributions

Conceptualization: V. M. and S. Ramanathan; data curation: S.-A. T. and A. P. B.; formal analysis: S.-A. T. and A. P. B.; funding acquisition: V. M. and S. Ramanathan; investigation: A. P. B; methodology: V. M., S. Ramanathan and S.-A. T.; project administration: V. M., S. Ramanathan and S.-A. T.; resources: V. M. and S. Ramanathan; software: S.-A. T.; supervision: V. M. and S. Ramanathan; validation: V. M., S.-A. T., A. P. B and N. A. K.; visualization: S.-A. T.; writing-original draft: S. A.-T., M. R. H., S. Rajoo and T. A.; writing-review and editing: S.-A. T., T. A., M. R. H., S. Rajoo, N. A. K., V. M. and S. Ramanathan.

Ethical committee clearance

Animal experimental procedures were approved by the Animal Ethics Committee of Universiti Sains Malaysia, Penang [approval number: USM/2015/ (678)]. Institutional guidelines were always adhered when handling the animals before and during the commencement of experiment.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Abatan, O.I.; Welch, K.B. and Nemzek, J.A.(2008). Evaluation of saphenous venipuncture and modified tail-clip blood collection in mice. J. Am. Assoc. Lab. Anim. Sci., **47**(3):8-15.

Afdhal, N.; McHutchison, J.; Brown, R.; Jacobson, I.; Manns, M.; Poordad, F.; Weksler, B. and Esteban, R.(2008). Thrombocytopenia associated with chronic liver disease. J. Hepatol., **48**(6):1000-1007.

Agarwal, T.; Tan, S.-A.; Onesto, V.; Law, J.X.; Agrawal, G.; Pal, S.; Lim, W.L.; Sharifi, E.; Moghaddam, F.D. and Maiti, T.K. (2021). Engineered herbal scaffolds for tissue repair and regeneration: Recent trends and technologies. Biomed. Eng. Adv., **2**:100015.

Ahmad, A.; Ibrahim, M.; Ansari, S. H.; Taleuzzaman, M. and Ahmad, S. (2021). Antithrombocytopenic potential of metabolically characterized extract and fractions of *Momordica charantia* L. fruits by *in vivo* study. Ann. Phytomed., **10**(2):264-272.

Anjum, V.; Arora, P.; Ansari, S.H.; Najmi, A.K. and Ahmad, S.(2017). Antithrombocytopenic and immunomodulatory potential of metabolically characterized aqueous extract of *Carica papaya* leaves. Pharm. Biol., **55**(1):2043-2056.

Bendran, A.P.(2016). Antibacterial, antioxidant and platelet boosting properties of Semalu and Manna Plus extracts. Msc.Thesis, Universiti Sains Malaysia, Penang.

Benzie, I.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem., **239**:70-76.

Bhattacharjee, S. and Banerjee, M.(2020). Immune thrombocytopenia secondary to COVID-19: A systematic review. SN Compr, Clin, Med., 1-11.

Biospektra (2021a). BiospektraSemalu. https://bio-spektra.com/product/biospektra-semalu/. (Assessed on 3 December 2021).

Biospektra (2021b). Biospektra Manna Plus. https://bio-spektra.com/product/biospektra-manna-plus/. (Assessed on 3 December 2021).

Blois, M.S.(1958). Antioxidant determinations by the use of a stable free radical. Nature, **181**:1199-1200.

Ekor, M.(2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front. Pharmacol., **4**:177.

Gadade, J. P. and Patil, S. A. (2019). Phytochemical paradigm, antioxidant status and their correlation in *Rotheca serrata* (L.) Steane and Mabb. Ann. Phytomed., **8**(2):156-166.

Gezici, S.; Koçum, D.; Yayla, F.; Sekeroglu, N. and Khan, A. (2020). Screening for *in vitro* antioxidant activities, polyphenolic contents and neuroprotective potentials of *Clinopodium serpyllifolium* subsp. serpyllifolium endemic to Turkey. Ann. Phytomed., **9**(1):181-186.

Huang, D.; Ou, B. and Prior, R.L.(2005). The chemistry behind antioxidant capacity assays. J. Agric. Food Chem., **54**:1841-1856.

Izak, M. and Bussel, J.B.(2014). Management of thrombocytopenia. F1000 Prime Rep., **6**:45.

Jabri Karoui, I. and Marzouk, B.(2013). Characterization of bioactive compounds in Tunisian bitter orange (*Citrus aurantium* L.) peel and juice and determination of their antioxidant activities. Biomed Res. Int., pp:345-415.

Jones, W.P. and Kinghorn, A.D.(2012). Extraction of plant secondary metabolites. Methods Mol. Biol., **864**:341-366.

Joris, I.; Braunstein, P.W.; Pechet, J.L. and Majno, G.(1980). Effect of thrombocytopenia on wound healing. A study in the rat. Exp. Mol. Pathol., **33**:283-291.

Kaur, C. and Kapoor, H.C.(2002). Antioxidant activity and total phenolic content of some Asian vegetables. Int. J. Food Sci. Technol., **37**: 153-161.

Kroyer, T.G.(2004). Red clover extract as antioxidant active and functional food ingredient. Innov. Food Sci. Emerg. Technol., **5**:101-105.

Liebman, H.A. (2014). Thrombocytopenia in cancer patients. Thromb. Res., **133**(2):S63-S69.

Malik, R.; Madan, V. and Prakash, R. (2020). Herbs that heal: Floristic boon to the natural healthcare system. Ann. Phytomed., **9**(2):1-9.

Manasa, K.; Soumya, R. and Vani, R.(2016). Phytochemicals as potential therapeutics for thrombocytopenia. J. Thromb. Thrombolysis., **41**(3):436-440.

Mivechi, N.F. and Ogilvie, P.D.(1989). Enhancement of murine bone marrow ablation by combining whole body hyperthermia with total body irradiation and cyclophosphamide. Int. J. Radiat. Oncol. Biol. Phys., **17**:117-123.

Nayak, D.; Chadha, V.; Jain, S.; Nim, P.; Sachdeva, J.; Sachdeva, G.; Vivekanand, K.; Khurana, A.; Raheja, S.M. and Manchanda, R.K. (2019). Effect of adjuvant homeopathy with usual care in management of thrombocytopenia due to dengue: a comparative cohort study. Homeopathy, **108**(3): 150-157.

Patil, S.; Shetty, S.; Bhide, R. and Narayanan, S.(2013). Evaluation of platelet augmentation activity of *Carica papaya* leaf aqueous extract in rats. J. Pharmacogn. Phytochem., **1**:57-60.

Prasad, K.N.; Yang, B.; Yang, S.; Chen, Y.; Zhao, M.; Ashraf, M. and Jiang, Y. (2009). Identification of phenolic compounds and appraisal of antioxidant and anti-tyrosinase activities from litchi (*Litchi sinensis*Sonn.) seeds. Food Chem., **116**:1-7.

Qader, S.W.; Abdulla, M.A.; Chua, L.S.; Najim, N.; Zain, M.M. and Hamdan, S.(2011). Antioxidant, total phenolic content and cytotoxicity evaluation of selected Malaysian plants. Molecules, **16**:3433-3443.

Qi, Q.; Dong, Z.; Sun, Y.; Li, S. and Zhao, Z.(2018). Protective effect of bergenin against cyclophosphamide-induced immunosuppression by immunomodulatory effect and antioxidation in Balb/c mice. Molecules, **23**(10):26-68.

Reagan-Shaw, S.; Nihal, M. and Ahmad, N. (2008). Dose translation from animal to human studies revisited. FASEB J., **22**:659-661.

Samson, M.; Fraser, W. and Lebowitz, D.(2019). Treatments for primary immune thrombocytopenia: A review. Cureus, **11**(10):e5849.

Sethumathi, P.; Manjuparkavi, K.; Vivekanandan, L.; Sivakumar, T.; Menaka, M.; Jayanthi, A. and Kumar, B. (2021). Evaluation of *in vitro* antioxidant and antimicrobial activity of polyherbal formulation of Thirikaduguchooranam and Parangipattaichooranam, Ann. Phytomed., **10**(2):169-174.

Sheng, Y.; Chen, Y.J.; Qian, Z.M.; Zheng, J. and Liu, Y.(2020). Cyclophosphamide induces a significant increase in iron content in the liver and spleen of mice. Hum. Exp.Toxicol., **39**(7):973-983.

Shimada, K.; Fujikawa, K.; Yahara, K. and Nakamura, T.(1992). Antioxidative properties of xanthone on the auto oxidation of soybean in cyclodextrin emulsion. J. Agric. Food Chem., **40**:945-948.

Soundravally, R.; Sankar, P.; Bobby, Z. and Hoti, S.L.(2008). Oxidative stress in severe dengue viral infection: Association of thrombocytopenia with lipid peroxidation. Platelets, **19**(6):447-454.

Subenthiran, S.;Choon, T.C.; Cheong, K.C.;Thayan, R.; Teck, M.B.;Muniandy, P.K.;Afzan, A.; Abdullah, N.R. and Ismail, Z.(2013).*Carica papaya* leaves juice significantly accelerates the rate of increase in platelet count among patients with dengue fever and dengue haemorrhagic fever. Evid. -Based Complement. Altern. Med., pp:1-7.

Wang, S.; Liu, Y.; Li, G.; Feng, Q.; Hou, M. and Peng, J.(2021). Reduced intracellular antioxidant capacity in platelets contributes to primary immune thrombocytopenia *via* ROS-NLRP3-caspase-1 pathway. Thromb. Res., **199**:1-9.

World Health Organization (WHO). (2011). Quality control methods for herbal materials. https://apps.who.int/iris/handle/10665/44479. (Assessed on 3 December 2021).

Yen, G.C.; Duh, P.D. and Tsai, C.L.(1993). Relationship between antioxidant activity and maturity of peanut hulls. J. Agric. Food Chem., **41**:67-70.

Citation **Sheri-Ann Tan, Akash Parthi Bendran, Mohammad Razak Hamdan, Nur Azzalia Kamaruzaman, Suresh Rajoo, Tarun Agarwal and Surash Ramanathan, Vikneswaran Murugaiyah (2022). Platelet boosting effect of commercial herbal supplements in chemically-induced thrombocytopenic rats. Ann. Phytomed., 11(1):276-282. http://dx.doi.org/10.54085/ap.2022.11.1.28.**