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Thrombocyte counts in mice after the administration of methanolic extract of *Melastoma malabathricum*

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PEER REVIEW

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Comments

This is a reasonably good study whereby the authors have revealed that the methanol leaf extract of *Melastoma malabathricum* was able to increase thrombocytes/platelets *in vivo*. It provides a preliminary data that could be used for further studies on bioassay-guided isolation of the active compound(s) and mechanistic action.

Details on Page 329

ABSTRACT

Objective: To investigate the effect of methanolic extract of *Melastoma malabathricum* (*M. malabathricum*) in thrombocyte counts in mice.

Methods: Methanolic extract of *M. malabathricum* corresponding to 1.5 to 2 mg/10 g body weight in saline was administered to mice via oral route. Control group was given normal saline. Twenty five microlitres of blood were drawn at 0 h and thereafter at 1, 2, 4, 8, 12, 20 h after dosing via tail bleeding technique. The thrombocytes were counted in the triple laminated middle 25 squares of the haemocytometer using light microscope.

Results: The control group showed a moderate rise after the administration of saline whereas the *M. malabathricum* treated group shows significant rise within 2nd h in thrombocyte counts with an increment of 51.64% compared to baseline count.

Conclusions: Based on the results, it can be concluded that *M. malabathricum* could be a potential remedy in treating thrombocytopenic condition.

KEYWORDS

Melastoma malabathricum, Thrombocytes, Thrombocytopenic condition**1. Introduction**

Melastoma malabathricum (*M. malabathricum*) is a plant from the family (Melastomaceae) commonly found in previously cleared land, waste places, and roadside throughout the Southeast Asian countries, including Malaysia[1]. *M. malabathricum* consists of three varieties, having flowers with dark purple–magenta petals, light pink–magenta petals and the rare variety of white petals[2]. There are a lot of uses for *M. malabathricum* reported in

folk medicine, but not supported by clinical data. Generally, various parts of the plant are used in Malay, Indian, and Indonesian folk medicines to treat various types of ailments and diseases including diarrhea, dysentery, leucorrhoea, haemorrhoids, cuts and wounds, infection during confinement and also used to prevent scarring of smallpox and to treat piles[3,4]. The most recent research on *M. malabathricum* revealed that it possesses antinociceptive, anti-inflammatory and antipyretic properties[5]. In addition, antioxidant activity of *M. malabathricum* was also

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reported^[6,7]. Despite being a traditional medicinal herb that is widely used, particularly, in Malay culture, there is not much scientific study carried out on *M. malabathricum*^[8]. This could limit the use of this plant as a potential medicinal remedy. In this study, we have investigated the effect of methanolic extract of *M. malabathricum* white petal variety on thrombocytes counts in mice.

2. Materials and methods

Methanolic extract of *M. malabathricum* leaves in saline was administered by gavage to 10 albino mice. Each mouse received 0.3–0.4 mL of the extract, corresponding to a dose of 1.5 to 2 mg/10 g body weight of the extract. A control group of 6 albino mice was given each orally normal saline at the same dose volume (0.3–0.4 mL). After dosing, blood sampling was done by using the standard tail bleeding technique. Twenty five microlitres of blood were drawn at 0 h and thereafter at 1, 2, 4, 8, 12, 20 h after dosing in all animals. Upon collection, the blood samples were transferred into test tube containing 475 μ L of ammonium oxalate diluents and vortex mixed immediately for 10 min. The haemocytometer filled with this mixture was incubated in a moist chamber for 10–20 min to allow the platelets to settle down. Round to oval shaped platelets were counted in the triple laminated middle 25 squares of the haemocytometer using light microscope (objective 40 \times and eyepiece 10 \times). (Compound light microscope (Olympus, CX21). The percentage changes of the thrombocyte count are determined using the following formula:

$$\% \text{ increase} = \frac{\text{Count after treatment} - \text{Count before treatment}}{\text{Count before treatment}} \times 100\%$$

The animal experiment was performed according to AIMST University's ethical committee approval (AUHAEC 47/FOP/2010) and guidelines. All the results obtained were expressed as mean \pm SD and statistical significant between treated and control groups was analyzed by means of Student's *t*-test using SPSS 13.0 for windows, where $P < 0.05$ was considered as statistically significant.

3. Results

The thrombocyte counts of the control group receiving normal saline and animals treated with methanolic extract of *M. malabathricum* is shown in Figure 1. The control group showed a moderate rise after the administration of saline; the platelet count peaked at 12 h with increment of 29.39%, which is less significantly different ($P > 0.05$) from the

baseline count (hour 0). As for the *M. malabathricum* treated group, the platelet count showed a statistically significant ($P < 0.05$) rise up to 2 h with an increment of 51.64% compared to baseline count. Comparing both control groups with the drug treated group of animals, there were significant differences ($P < 0.05$) between the platelet counts from 0 h to 4 h continuously. The rise in the platelet count in control could be either the expression of a natural circadian cycle of thrombocyte density. The result showed that there is a significant increase of platelet count after administration of methanolic extract of *M. malabathricum* compared to control group.

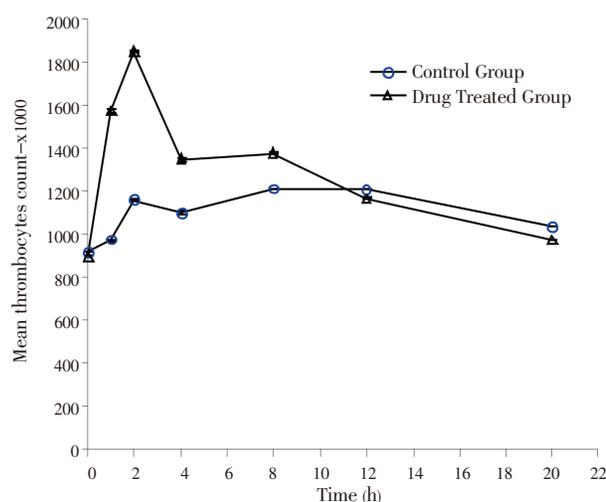


Figure 1. Graph of thrombocyte counts against time.

4. Discussion

The thrombocytes increasing effect of the oral administration of freshly prepared methanolic extract of *M. malabathricum*, evident from the present study, does not directly pinpoint to an exact mechanism/s of action. However, under normal healthy body conditions, platelets are produced from megakaryocytes within 4 to 6 d in humans and 2–3 d in rodents^[9]. Nevertheless, in this study, a dramatic increase of platelets was observed within only 2 h after administration of *M. malabathricum*. It is reported that under normal healthy body conditions spleen tends to hold one third of the platelets produced by megakaryocytes^[10]. The smooth muscle contraction of the spleen, releases stored platelets in to the circulation. Therefore, the platelet increasing effect of the *M. malabathricum* could be due to either megakaryopoietic/thrombopoietic stimulatory activity, and or by induced spleen contractions. A possible synergistic effect of these two mechanisms also can't be ruled out in increasing the platelet count. The results call for a dose–response investigation and subsequently extending the studies to isolate and identify the bioactive compounds

from *M. malabathricum* which are responsible for the release and/or production of thrombocytes. In conclusion, the *M. malabathricum* could be a potential remedy in treating thrombocytopenic condition.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Thrombocytopenia is a condition of abnormally low platelet count in the blood. Platelets play an important function in blood clotting formation. Thrombocytopenia is often associated with bleeding problems.

Research frontiers

The present research work provided new preliminary scientific knowledge on the ability of *M. malabathricum* leaf extract to increase thrombocytes in vivo.

Related reports

M. malabathricum has been reported to possess antioxidant, antibacterial, cytotoxic, antiinflammatory, antinociceptive, antipyretic, antidiarrhoeal, antiulcer, gastroprotective, wound healing, hepatoprotective, antidiabetic, antihyperlipidemic, immunomodulatory and male fertility enhancement activities. Thus, this research adds another scientific evidence of its benefits.

Innovations and breakthroughs

M. malabathricum leaves are traditionally been used for diarrhea, dysentery, after childbirth and wound healing. In the present study, the authors have demonstrated that the methanol extract increased platelet count that could be linked to the prevention of bleeding problems.

Applications

The *in vivo* study may suggest the potential application

of *M. malabathricum* leaf extract for thrombocytopenic condition and the extract could be further developed as standardized herbal product. Previous acute toxicological study reported that the methanol leaf extract had LD₅₀ value of more than 5000 mg/kg.

Peer review

This is a reasonably good study whereby the authors have revealed that the methanol leaf extract of *M. malabathricum* was able to increase thrombocytes/platelets *in vivo*. It provides a preliminary data that could be used for further studies on bioassay-guided isolation of the active compound(s) and mechanistic action.

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