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# Thrombocyte counts in mice after the administration of papaya leaf suspension

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## Thrombozytenzahl bei Mäusen nach Verabreichung einer Papaya-Blattsuspension

**Zusammenfassung.** Angeregt von dem volksmedizinischen Gebrauch von *Papaya carica* Blattmaterial zur Behandlung von Dengueinfektionen wurde eine Suspension von pulversisierten *Carica papaya* Blättern in Palmöl auf die Beeinflussung der Thrombozytenzahlen bei Mäusen untersucht. Jeweils 5 Mäuse erhielten peroral 15 mg der *Carica papaya* Blattsuspension, gleiche Volumina physiologischer Kochsalzlösung oder Palmöl. Die Thrombozytenzahl wurde unmittelbar vor sowie 1, 2, 4, 8, 10, 12, 24, 48 und 72 Stunden nach der Applikation bestimmt. Bei den mit *C. papaya* behandelten Mäusen zeigte sich eine signifikante Erhöhung der Thrombozytenzahl 1, 2, 4, 8, 10 und 12 Stunden nach der Anwendung. Die mit physiologischer Kochsalzlösung behandelte Gruppe zeigte lediglich eine zeitweilige, nicht signifikante Erhöhung der Thrombozytenzahlen, offenbar Ausdruck eines zirkadischen Zyklus. In der mit Palmöl allein behandelten Gruppe wurde eine protrahierte, bei 8 und 48 Stunden signifikante Erhöhung der Thrombozytenzahl verzeichnet, möglicherweise als Folge einer bisher unbekannten Stimulation der Freisetzung von Thrombozyten. Die Ergebnisse legen eine erweiterte Dosisstudie nahe, sowie die Isolierung und strukturelle Aufklärung der für die Ausschüttung bzw. Produktion von Thrombozyten verantwortlichen Inhaltsstoffe von *C. papaya*.

**Summary.** Following up a popular use of crude leaf preparations from *Carica papaya* for the treatment of dengue infections, a suspension of powdered *Carica papaya* leaves in palm oil has been investigated for its effect on thrombocyte counts in mice, administering by gavage 15 mg of powdered leaves per kg body weight to 5 mice. Equal numbers of animals received corresponding volumes of either palm oil alone or physiological saline solution. Thrombocyte counts before and at 1, 2, 4, 8, 10, 12, 24, 48 and 72 hours after dosing revealed significantly higher mean counts at 1, 2, 4, 8, 10 and 12 after dosing with the *C. papaya* leaf formulation as compared to the mean count at hour 0. There was only a non-significant rise of thrombocyte counts in the group having received saline solution, possibly the expression of a normal circadian rhythm in mice. The group having received palm oil only showed a protracted increase of platelet counts that was significant at hours 8 and 48 and obviously the result of a hitherto unknown stimulation of thrombocyte release. The results call for a dose-response investigation and for extending the studies to the isolation and identification of the *C. papaya* substances responsible for the release and/or production of thrombocytes.

**Key words:** *Carica papaya*, *Mus musculus*, thrombocytes, Dengue.

## Introduction

*Carica papaya* L., belongs to the plant family *Caricaceae.* It is being cultivated widely for consumption as fresh fruit, dried and crystallised fruit as well as for use in drinks, jams and candies [1]. Green fruit, the leaves and flowers may also be used as a cooked vegetable [2]. Nakasone and Paull [3] have shown that papaya is a good source of calcium and an excellent source of vitamins A and C. Papaya also has several industrial uses. Biochemically, its leaves and fruit are complex, containing several proteins and alkaloids with important pharmaceutical and industrial applications [4]. Commonly, *Carica papaya* is used as food or as medication in folk medicine [5]. Considerable work has been carried out on plant parts such as fruit, seed and root, indicating the presence of biologically active compounds. The quantity of the compounds differs in fruit, latex, leaves and roots, and varies with the extraction method, age of the plant part, the cultivation and the gender of the tree.

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The activity of *Carica papaya* root extract in rats suggests a purgative effect mediated via the cholinergic system. A compound present in crushed papaya seed that is believed to have activity against helminthic intestinal parasites, benzyl isothiocyanate, has been shown to have an effect on vascular contraction using a canine carotid artery in vitro model [6]. Further reports point to an antihypertensive activity of papaya fruit extracts [5]. Other investigations indicate that *Carica papaya* fruit extract possesses antibacterial [7], antioxidant [8] and anti-inflammatory [9] activity. It is also used in the treatment of chronic skin ulcer and as a diuretic [10]. Research on *Carica papaya* seed extract concerns mainly antifertility properties [11–13]. The oral administration of *C. papaya* seed extract can induce reversible male infertility [12]. Administration of the benzene chromatographic fraction of the chloroform extract of the seeds of *C. papaya* at a dose of 10 mg/rat/day for 150 days showed a total inhibition of sperm motility, reduced sperm count and infertility [11]. Apart from the effect on male rats, *C. papaya* seed extract manifested antifertility, antiimplantation and abortifacient activity in female rats as well. Some reports indicate that latex of *C. papaya* has antimicrobial and antiparasitic activity. Papaya latex was shown to be effective in killing *Ascaris suum* in pigs [14] and *Heligmosomoides polygyrus* infections in mice [15].

However, little work has been done on *C. papaya* leaves. Diseases such as dengue, idiopathic thrombocytopenic purpura, malignancy, hypersplenism, and aplastic anaemia result in a low thrombocyte count in the blood. Rapid response to thrombocyte and fresh frozen plasma transfusion was observed in cases of dengue virus infection [16]. Recently, crude formulations of papaya leaf have been successfully employed in folk medicine in continental Malaysia for the treatment of dengue infections with haemorrhagic manifestations, using suspensions of powdered leaves in palm oil, a popular vehicle in local herbal medicine. This use gave rise to an investigation on the effect of *C. papaya* crude leaf material on the thrombocyte count in an animal model, the more so as the scientific literature is devoid of relevant reports.

## Material and methods

### *Mice (*Mus musculus*)*

Fifteen white male mice of the species *Mus musculus* (Swiss albino strain) were obtained from the animal house. The mice were housed in cages (5 per cage) and provided with water and food *ad libidum*. The cage bed consisted of sawdust and was changed every three days to maintain good hygiene. The weight of the mice ranged from 27 g to 37 g.

### *Chemicals and apparatus*

Ammonium oxalate was purchased from Ajax Chemicals, Australia. Palm oil was obtained from the local market. A haemocytometer (Neubauer, Assistant, Germany) and a light microscope (Nikon, Model YS 100, Japan) were used for the thrombocyte counts.

### *Plant material*

Raw, mature and fully green *Carica papaya* leaves from male trees were collected at the residence of Bandar Laguna Merbok, Sungai Petani, State of Kedah, Malaysia. The *Carica papaya* leaves were washed with water to remove dirt prior to the drying process. The leaves were dried and shredded into small pieces by using a scissor. The dried leaf material was ground into a fine powder using an electric grinder, and weighed before being kept in a container held at room temperature.

### *Preparation of* Carica papaya *leaves powder suspension*

Fifty milligrams of dried and pounded crude *Carica papaya* leaves was weighed and suspended in 50 ml of palm oil. The suspension was sonicated for 30 minutes in a water bath set at 37°C.

### *Animal study*

A suspension of powdered crude *Carica papaya* leaves in palm oil was administered, by gavage, to five mice. Each mouse received 0.405–0.555 ml of the suspension, corresponding to a dose of 15 mg/kg

**Table 1.** Mean thrombocyte counts in three groups of 5 mice each, having received physiological saline or palm oil as controls, or ground leaves of *C. papaya* in palm oil [*t* (Student) and *p* = comparison to hour 0 count]

| Hour | Saline controls | | | Palm oil controls | | | Papaya leaf formulation | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean count $\times 10^3$ | *t* | *p* | Mean count $\times 10^3$ | *t* | *p* | Mean count $\times 10^3$ | *t* | *p* |
| 0 | 847.3 | – | – | 899.1 | – | – | 948.0 | – | – |
| 1 | 869.5 | 0.0229 | >0.05 | 1123.0 | 1.0243 | >0.05 | 1788.0 | 3.6363 | 0.0066 |
| 2 | 1063.9 | 0.8063 | >0.05 | 1488.8 | 1.6320 | >0.05 | 2483.0 | 8.1756 | $3.05 \times 10^{-5}$ |
| 4 | 1130.0 | 1.2650 | >0.05 | 1330.8 | 1.6687 | >0.05 | 3024.5 | 10.0025 | $5.59 \times 10^{-6}$ |
| 8 | 1505.0 | 1.9861 | >0.05 | 1256.0 | 2.4847 | 0.0377 | 2698.6 | 3.6394 | 0.0066 |
| 10 | 1356.4 | 1.7316 | >0.05 | 1570.2 | 1.6338 | >0.05 | 1805.0 | 2.0402 | 0.0186 |
| 12 | 1322.4 | 1.8455 | >0.05 | 1732.8 | 1.6291 | >0.05 | 2178.5 | 5.1790 | $8.39 \times 10^{-4}$ |
| 24 | 1287.8 | 1.8333 | >0.05 | 1135.9 | 0.9153 | >0.05 | 1260.5 | 1.3765 | >0.05 |
| 48 | 1003.0 | 0.6188 | >0.05 | 1284.9 | 2.6884 | 0.0274 | 1307.5 | 1.3374 | >0.05 |
| 72 | 895.5 | 0.1027 | >0.05 | 959.2 | 0.4045 | >0.05 | 1198.5 | 0.8024 | >0.05 |

of ground papaya leaves. A control group ($n=5$) was given the vehicle (palm oil) alone at the same dose volume. Another five mice received physiological saline at the same dose volume. After dosing, blood sampling was done by using the standard tail bleeding technique [17]. Twenty five microlitres of blood were drawn at 0 hr and thereafter at 1, 2, 4, 8, 10, 12, 24, 48, and 72 hours after dosing in all animals. Upon collection, the blood samples were transferred into test tubes containing 475 µl of ammonium oxalate diluent. The mixture was vortex-mixed for 15 minutes. The study protocol has been approved by the Animal Ethics Committee, Universiti Sains Malaysia.

### *Platelet count*

Blood-diluent mixture (60 µl) was transferred onto the Neubauer counting chamber using a micropipette. The counting chamber was placed in a moist Petri dish and left standing untouched for at least 20 minutes to ensure the settling of the platelets. The preparation was examined under a light microscope (objective 40× and eyepieces 10×). The number of thrombocytes in 1 ml of blood was calculated using the formula of Dacie and Lewis [18]. Subsequently it was adjusted to the number of thrombocytes in 1 µl.

## Results and discussion

The thrombocyte count in the control animals having received physiological saline (Table 1, Figs. 1, 3) showed a moderate, statistically not significant rise of the platelet counts peaking at 8 hours. At no time between 1 and 72 hours after the administration of the saline solutions were the thrombocyte counts significantly different from the baseline count (hour 0).

The thrombocyte counts in the control mice having received palm oil showed a similar rise as that observed in the saline controls, though with the peak at 12 hours (Table 1, Fig. 3). The peak count did not reach the threshold of statistical significance.

In the mice having received the ground-leaf suspension of *C. papaya* in palm oil, the thrombocyte counts showed a statistically significant rise that lasted, without interruption, from 1 to 12 hours after having received the formulation, with a peak at 4 hours (Table 1, Figs. 2, 3). The thrombocyte counts before and 72 hours after the administration of the formulations (hours 0 and 72) were quite similar (Fig. 3). Comparing the thrombocyte counts observed in the saline and palm oil controls (Table 2), no significant differences were observed, except at 48 hours ($t=2{,}4437$; $p=0.0402$) when the count in the palm oil controls was higher than that in the saline controls. Comparing the thrombocyte counts in the saline controls with those observed in the animals having received the papaya-leaf formulation, the counts in the latter were higher than those in the control mice at hours 1, 2, 4, 8, 12, 48 and 72 after the administration of the formulations. Similarly, the comparison of the thrombocyte counts in the palm oil controls and those in the animals having received the papaya-leaf formulation yielded higher counts in the latter from hours 1 to 72 after the administration of the formulations.

As to the temporary rise of the platelet counts during the early observation phase in the saline and palm oil control animals, this could be the expression of a natural circadian cycle of thrombocyte density. Nevertheless, the generally higher platelet counts in the palm oil controls as compared to the saline controls seem to indicate a hitherto unknown stimulation of thrombocyte release that reached significance at 48 hours after

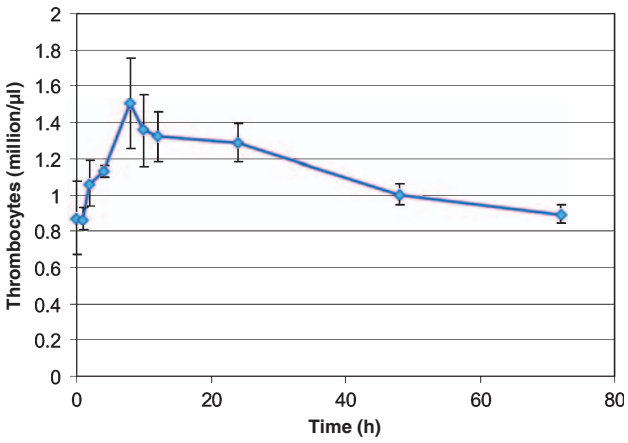


**Fig. 1.** Mean thrombocyte counts in control mice having received physiological saline solution (Standard errors indicated above the data points)

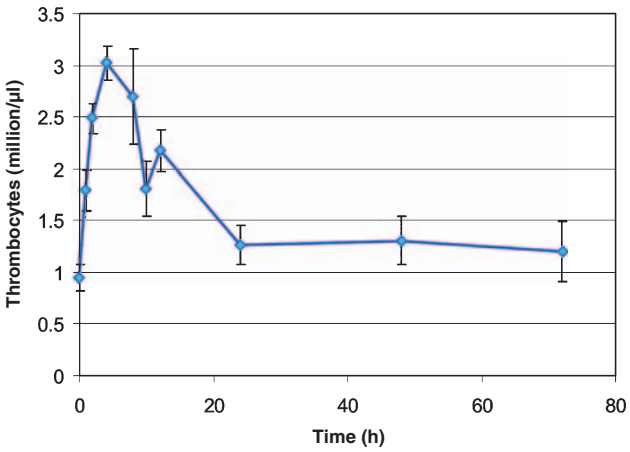


**Fig. 2.** Mean thrombocyte counts in mice having received the *Carica papaya* leaf formulation (standard errors indicated above the data points)

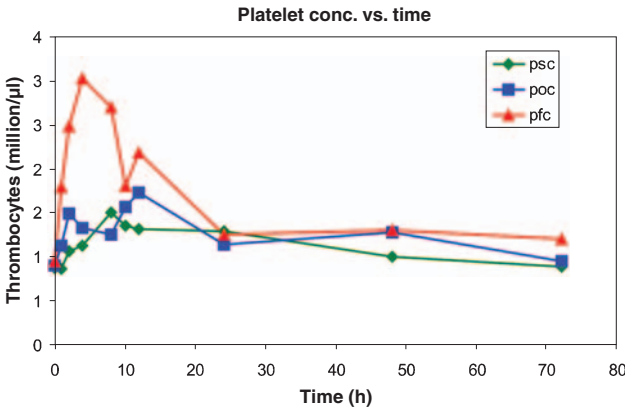


**Fig. 3.** Mean thrombocyte counts in control mice having received either physiological saline solution (psc) or palm oil (poc) and in mice having received the *Carica papaya* leaf formulation (pfc)

**Table 2.** Results of Student's t-test for the comparison between saline controls, palm oil controls and animals having received *C. papaya* leaf formulation

| Hour | Saline vs. papaya form. | | Palm oil vs. papaya form. | | Saline vs. palm oil controls | |
|---|---|---|---|---|---|---|
| | *t* | *p* | *t* | *p* | *t* | *p* |
| 0 | 0.3131 | >0.05 | 0.3018 | >0.05 | 0.1103 | >0.05 |
| 1 | 4.5055* | 0.0020 | 2.4300* | 0.0411 | 1.2541 | >0.05 |
| 2 | 7.5712* | $5.6\times10^{-5}$ | 2.6613* | 0.0286 | 1.1551 | >0.05 |
| 4 | 11.2146* | $2.0\times10^{-6}$ | 5.8516* | 0.00037 | 0.8392 | >0.05 |
| 8 | 2.2673 | >0.05 | 3.0356* | 0.0161 | 0.9349 | >0.05 |
| 10 | 1.3710 | >0.05 | 0.4923 | >0.05 | 0.4833 | >0.05 |
| 12 | 3.4954* | 0.0081 | 0.8247 | >0.05 | 0.7894 | >0.05 |
| 24 | 0.1259 | >0.05 | 0.4103 | >0.05 | 0.5857 | >0.05 |
| 48 | 1.2421 | >0.05 | 0.6876 | >0.05 | 2.4437** | 0.0402 |
| 72 | 1.0411 | >0.05 | 0.7835 | >0.05 | 0.5349 | >0.05 |

* Higher thrombocyte count with papaya formulation
** Higher thrombocyte count with palm oil control

the administration of the formulations. Despite the initial uptrend of the platelet counts in the control groups, the consistent rise of the thrombocyte counts in the group treated with the papaya-leaf formulation was higher and significant in comparison, reaching p values as low as 0.000002, indicating a potential of papaya leaf formulations in the treatment of thrombocytopenic purpura. This therapeutic potential should be further investigated by a dose response experiment and by isolating and identifying the substances responsible for the release and/or production of the thrombocytes, followed by studies based on the use of the pure substances in suitable formulations.

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

## References

1. Villegas VN (1997) *Carica papaya* L. In: Coronel RE, Verheij EWM (eds) Edible fruits and nuts. Wageningen University, The Netherlands, vol 1, p 447
2. Watson B (1997) Agronomy/agroclimatology notes for the production of papaya. MAFFA, Australia, p 187
3. Nakasone HY, Paull RE (1998) Tropical fruits. CAB International, Wallingford, UK, p 353
4. El Moussaoui A, Nijs M, Paul C, Wintjens R, Vincentelli J, Azarkan M, Looze Y (2001) Revisiting the enzymes stored in the lactifers of *Carica papaya* in the context of their possible participation in the plant defence mechanism. Cell Mol Life Sci 58: 556–70
5. Eno AE, Owo OI, Itam EH, Konya RS (2000) Blood pressure depression by the fruit juice of *Carica papaya* (L.) in renal and DOCA-induced hypertension in the rat. Phytother Res 14: 235–9
6. Wilson RK, Kwan TK, Kwan CY, Sorger GJ (2002) Effects of papaya seed extract and benzyl isothiocyanate on vascular contraction. Life Sci 71: 497–507
7. Emeruwa AC (1982) Antibacterial substance from *Carica papaya* fruit extract. J Nat Prod 45: 123–7
8. Rimbach G, Guo Q, Akiyama T, Matsugo S, Moini H, Virgili F, Packer L (2000) Ferric nitriloacetate induced DNA and protein damage: inhibitory effect of a fermented papaya preparation. Anticancer Res 20: 2907–14
9. Gupta OP, Sing S, Bani S, Sharma N, Malhotra S, Gupta BD, Banerjee SK, Handa SS (2000) Anti-inflammatory and anti-arthritic activities of silymarin acting through inhibition of 5-lipoxygenase. Phytomedicine 7: 21–4
10. Hewitt HH, Whittle S, Lopez SA, Bailey EY, Weaver SR (2000) Topical use of papaya in chronic skin ulcer therapy in Jamaica. West Indian Med J 49: 32–3
11. Manivannan B, Mishra PK, Pathak N, Sriram S, Bhande SS, Panneerdoss S, Lohiya NK (2004) Ultrastructural changes in the testis and epididymis of rats following treatment with the benzene chromatographic fraction of the chloroform extracts of the seed of *Carica papaya*. Phytother Res 18: 285–9
12. Udoh O, Essien I, Udoh F (2005) Effects of *Carica papaya* (paw paw) seeds extract on the morphology of pituitary-gonadal axis of male Wistar rats. Phytother Res 19: 1065–8
13. Chinoy NJ, D'Souza JM, Padman P (1994) Effects of crude aqueous extract of *Carica papaya* seeds in male albino mice. Reprod Toxicol 8: 75–9
14. Satrija F, Nansen P, Murtini S, He S (1995) Anthelminthic activity of papaya latex against patent *Heligmosoides polygyrus* infections in mice. J Ethnopharmacol 48: 161–4
15. Satrija F, Nansen P, Björn H, Murtini S, He S (1994) Effect of papaya latex against *Ascaris suum* in naturally infected pigs. J Helminthol 68: 343–6
16. World Health Organization (1997) Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. 2nd edn. WHO, Geneva, p 31
17. Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal JM, Vorstenbosch CVD (2001) A good practice guide to the administration of substances and removal of blood, including routes and volumes. J Appl Toxicol 21: 15–23
18. Lewis SM, Bain BJ, Bates I (eds) (2006) Dacie and Lewis practical haematology. Elsevier, UK, p 595