



## Analgesic activity of ethanolic extract of *Manihot esculenta* Crantz leaves in mice

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### ABSTRACT

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Cassava (*Manihot esculenta* Crantz) leaves have long been used as a vegetable in many countries and empirically as a febrifuge. The aim of the present study was to evaluate the analgesic activity of an ethanolic extract of cassava leaves in mice. Thirty Balb/c mice (20-30 g, 2-3 months old) were randomly divided into 6 groups. Group I was given distilled water 1 mL as negative control, group II paracetamol 65 mg/kgBW as positive control, and group III-VI received an ethanolic extract of cassava leaves in 4 doses, i.e. 12.8 mg/kgBW, 25.6 mg/kgBW, 51.3 mg/kgBW, and 102.6 mg/kgBW, respectively. All interventions were administered as a single dose by oral route on a given day. Acetic acid 0.6% (w/v) was used as a pain inductor. Analgesic activity was measured by counting the percentage of writhing movements as a measure of the analgesic effect produced by each intervention. Data were analyzed with one-way Anova to compare analgesic activity between treatment groups. The results showed that groups treated with ethanolic extract of cassava leaves at dosages of 12.8 mg/kgBW, 25.6 mg/kgBW, 51.3 mg/kgBW, and 102.6 mg/kgBW had an analgesic activity of 59.2%; 73.9%; 62.1%; and 55.9%, respectively. On statistical analysis there were significant differences ( $p=0.00$ ) between these treatments compared to the negative control, but no significant differences ( $p>0.05$ ) with the positive control (paracetamol). It may be concluded that the analgesic effect of an ethanolic extract of cassava (*Manihot esculenta* Crantz) leaves in mice was of similar potency as paracetamol.

**Keywords:** *Manihot esculenta* Crantz, analgesic activity, flavonoid, mice

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### INTRODUCTION

The cassava plant (*Manihot esculenta* Crantz, family Euphorbiaceae) is one of the staple food crops in most regions of Africa, Asia, and Latin America.<sup>(1)</sup> The parts of the plant that are commonly utilized are the roots

and leaves. In Indonesia cassava roots are used as an alternative staple food and cassava meal (tapioca flour) as a wheat flour substitute in the preparation of bread and cookies.<sup>(2)</sup> Cassava leaves have also been used against many disorders, such as rheumatism, fever, headache, diarrhea, and loss of appetite.<sup>(3,4)</sup> Cassava

leaves reportedly also possess antihemorrhoid, anti-inflammatory,<sup>(5)</sup> and antimicrobial activity.<sup>(6)</sup> In Nigeria, cassava is used for the treatment of ringworm, tumor, conjunctivitis, sores and abscesses. To date there are very few reports of studies on the analgesic activity of the cassava plant. A study in Nigeria showed that oral administration of an aqueous cassava leaf extract to rats induced anti-inflammatory and analgesic effects.<sup>(8)</sup>

The cassava plant is rich in macro- and micronutrients<sup>(1,9)</sup> and is therefore commonly used as a staple food. In addition the plant contains a number of antioxidant compounds, namely  $\beta$ -carotene (around 23-86 mg/100g),<sup>(7)</sup> vitamin C (1.7-419 mg/100g),<sup>(1)</sup> vitamin A, anthocyanins (flavonoids), saponins, steroids, and glycosides.<sup>(7,10)</sup> Previous studies have reported that several saponin- and flavonoid-containing plants possess analgesic, anti-inflammatory, antipyretic, and antimicrobial activities. These plants include *mahkota dewa* (*Phaleria macrocarpa*),<sup>(11)</sup> *kayu ulin* (*Eusideroxylon zwageri*) T et B,<sup>(12)</sup> star fruit (*belimbing manis*, *Averrhoa carambola* Linn.),<sup>(13)</sup> *daun bangun-bangun* (*Coleus amboinicus* L.),<sup>(14)</sup> and *Curcuma alismatofolia* Gagnep.<sup>(15)</sup> The flavonoid fraction and volatile flavonoid compounds of these plants are thought to have anti-inflammatory and analgesic effects.<sup>(16)</sup>

In addition to beneficial chemical compounds, cassava leaves also contain toxic substances, which are associated with the high concentration of cyanogenic glycosides. These are carbohydrate derivatives that release hydrocyanic acid upon hydrolysis. Hydrocyanic acid inhibits the reduction of oxygen in the (mammalian) respiratory system, the electron transport in photosynthesis, and the activity of enzymes such as catalase and oxidase, which play a role in transport of iodine in cases of goiter, although one study did not find evidence for a relationship between cassava consumption and the prevalence of goiter.<sup>(17)</sup>

The cyanogenic glycoside content depends to a great extent on the age and cultivar of cassava and on environmental factors.<sup>(18)</sup> Younger plants tend to have a higher cyanogenic glycoside content and the mature leaves on the lower part of the plant have a lower cyanogenic glycoside content.<sup>(2)</sup> Therefore in the present study only mature leaves were collected for preparation of the extract.

Since cassava leaves have a high level of antioxidants, such as flavonoids, saponins, vitamin C, and  $\beta$ -carotenes, the cassava plant is presumably capable of counteracting the effects of oxidants (free radicals), suggesting the possibility that the leaves affect the inflammatory process, the pathophysiology of which also involves free radicals. The aim of the present study was to evaluate the analgesic effect of an ethanolic extract of cassava leaves (*Manihot esculenta* Crantz) in mice.

## METHODS

### Research design

This was a pure experimental controlled laboratory study with the objective of evaluating the analgesic activity of an ethanolic extract of cassava (*Manihot esculenta* Crantz) leaves.

### Plant material

Cassava (*Manihot esculenta* Crantz) leaves were collected in January 2010 in the hamlet (*dusun*) of Ngalangan in Jetisbaran village, Ngaglik subdistrict, Sleman district, Yogyakarta province. The collection procedure selected the ripe leaves growing near the ground level, in order to obtain leaves with a minimal content of cyanogenic glycosides. Identification of the cassava leaves was performed at the Pharmacognosy-Phytochemistry Laboratory, Pharmacy Program, Faculty of Mathematics and Natural Sciences (FMIPA), UII, Yogyakarta, which also supplied the chemicals used in the preparation of the ethanolic extract. Material for assessment of analgesic activity was obtained

from the Pharmacology Laboratory of abovementioned faculty.

### **Extract preparation**

Cassava leaf ethanolic extract was prepared by maceration as follows: One kilogram of cassava leaves was rinsed in water, cut up into small pieces, then left to dry in a drying cabinet at 38°C for 5 days, before undergoing maceration. Subsequently the leaves were pulverized by means of a pestle-and-mortar and an electric blender to yield a dry powder of around 200 g in weight. For maceration, 100 g dry cassava leaf powder was put in an erlenmeyer flask, then 500 mL 70% ethanol was added, after which the mixture was left standing at room temperature for 24 hours. The leaf powder was subjected three times to the maceration process. The resulting solution (ethanol phase) was filtered and concentrated in an evaporator at 50°C to yield a concentrated ethanolic cassava leaf extract of about 10 g in weight.

### **Experimental animals**

This study used thirty Balb/c mice aged 2-3 months and weighing 20-30 g. The experimental animals were healthy and in excellent general condition, as was evident from their active movements and their black and round fecal pellets without signs of diarrhea.

### **Oral administration of *Manihot esculenta* Crantz**

Thirty mice were randomly allocated to six intervention groups comprising 5 mice each. Group I animals were given distilled water 1 mL and served as negative controls, while group II mice received paracetamol 65 mg/kgBW and served as positive controls. The dosages of paracetamol were based on dosages for human adults, using a conversion factor of 0.026. Groups III-VI were given 1 mL of ethanolic cassava leaf extract in dosages of 12.8 mg/kgBW, 25.6 mg/kgBW, 51.3 mg/kgBW, and 102.6 mg/kgBW, respectively. The lower dose

of 12.8 mg/kgBW was equivalent to a dose of dried cassava leave powder of 1 g/kgBW. This dosage was determined in preliminary studies from consideration of effective doses of previous studies. All interventions were administered on a given day as a single dose by gavage.

### **Acetic acid induced writhing in mice**

As pain inducer 0.6% (w/v) acetic acid was given by intraperitoneal injection in dosages of 10 mL/kgBW.<sup>(19)</sup> The resulting pain was inferred from the writhing movements of the mice, which were monitored at intervals of five minutes, for up to one hour for each experimental animal, yielding the cumulative total of writhing movements per hour.

### **Calculation of analgesic power**

Analgesic power was calculated on the basis of the inhibitory capacity of the interventions on the writhing movements of the experimental animals,<sup>(20)</sup> as follows:  
$$\% \text{ writhing protection} = 100 - [(E/C) \times 100]$$
where E = cumulative total of writhing in the experimental animals after intervention, and C = cumulative total of writhing in the negative controls.

A medication is said to have analgesic activity if it is capable of effecting a reduction of > 50% in the number of writhings of the experimental animals as compared to the control group.<sup>(20)</sup> To determine analgesic power, the percentage reduction in cumulative total number of writhings is calculated against the negative control group. The calculated percentage is termed the analgesic power of cassava leaf ethanolic extract against the pain induced by acetic acid stimulation of the experimental animals.

### **Statistical analysis**

Percentages of writhing protection in the intervention groups were analyzed by means of a one-way Anova, at significance level of  $p < 0.05$ .

Table 1. Number of writhings in mice after intervention and induction of pain by 0.6% acetic acid

| Intervention group                              | Cumulative number of writhings in mice |      |      |      |      | Mean $\pm$ SD    |
|---|--|------|------|------|------|------------------|
|   | 15'                                    | 210' | 315' | 420' | 525' |                  |
| Distilled water 1 mL<br>(negative control)      | 40                                     | 78   | 71   | 127  | 133  | 89.8 $\pm$ 39.44 |
| Paracetamol 65 mg/kgBW<br>(positive control)    | 17                                     | 24   | 35   | 46   | 44   | 33.2 $\pm$ 12.56 |
| Cassava leaf ethanolic extract<br>12.8 mg/kgBW  | 27                                     | 36   | 13   | 46   | 61   | 36.6 $\pm$ 18.26 |
| Cassava leaf ethanolic extract<br>25.6 mg/kgBW  | 7                                      | 11   | 20   | 46   | 33   | 23.4 $\pm$ 16.10 |
| Cassava leaf ethanolic extract<br>51.3 mg/kgBW  | 28                                     | 31   | 24   | 39   | 48   | 34 $\pm$ 9.56    |
| Cassava leaf ethanolic extract<br>102.6 mg/kgBW | 23                                     | 33   | 26   | 34   | 82   | 39.6 $\pm$ 24.15 |

## RESULTS

In preliminary acute toxicity tests, monitoring was performed on clinical signs considered to be representative of autonomic, behavioral, sensory, neuromuscular, cardiovascular, and respiratory systems. The tests involved four test animals, each of them receiving one of the four different dosages of the extract to be used in the present study. In these acute toxicity tests, all test animals failed to display clinical signs of toxicity, from which it was concluded that the four administered dosages could safely be used for the actual analgesic tests.

In the present study all test animals (n = 30) completed the study, as none of them died or suffered from toxic effects. The number of writhings of all test animals and mean cumulative total of writhings in the intervention groups are presented in Table 1 and Figure 1, respectively.

From Table 1 and Figure 1 it may be seen that there was a reduction in the cumulative total of writhings in the ethanolic extract groups and the paracetamol group, in comparison with the negative controls receiving distilled water. This indicates that paracetamol and cassava leaf ethanolic extract at the four dosages were capable of reducing

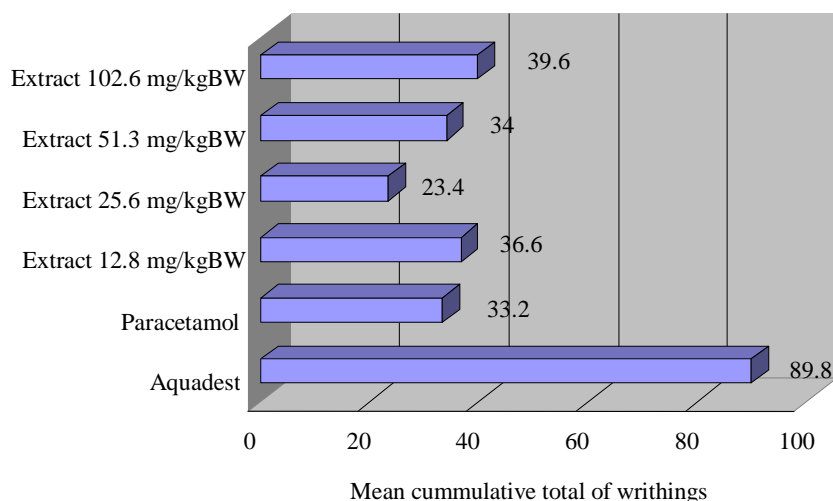


Figure 1. Mean cumulative total of writhings in intervention groups

Table 2. Percentage of writhing protection of paracetamol and cassava leaf ethanolic extract on pain in mice induced by 0.6% acetic acid

| Intervention group                              | Writhing protection (%) after intervention<br>(observed every 5 min.) |       |       |       |       | Mean ± SD     |
|---|---|-------|-------|-------|-------|---------------|
|   | 1   | 2     | 3     | 4     | 5     |               |
| Paracetamol<br>65 mg/kgBW                       | 81.07   | 73.27 | 61.02 | 45.78 | 51    | 63.03 ± 13.98 |
| Cassava leaf ethanolic extract<br>12.8 mg/kgBW  | 69.93   | 59.91 | 85.52 | 48.78 | 32.07 | 59.24 ± 20.33 |
| Cassava leaf ethanolic extract<br>25.6 mg/kgBW  | 92.2  | 87.75 | 77.73 | 48.78 | 63.25 | 73.94 ± 17.93 |
| Cassava leaf ethanolic extract<br>51.3 mg/kgBW  | 68.82   | 65.48 | 73.27 | 56.57 | 46.55 | 62.14 ± 10.65 |
| Cassava leaf ethanolic extract<br>102.6 mg/kgBW | 73.49   | 63.25 | 71.05 | 62.14 | 8.69  | 55.9 ± 26.89  |

Note: Negative control group: writhing protection = 0%

the occurrence of writhing movements as a response to the pain induced by intraperitoneal injection of 0.6% acetic acid.

The analgesic power (%) of cassava leaf ethanolic extract at several dosages on the writhing reflex induced by intraperitoneal acetic acid is presented in Table 2, while the percentage of writhing protection in the intervention groups is shown graphically in Figure 2. It is apparent from both Table 2 and Figure 2 that of the original six groups there are now only five, because the analgesic power

of the negative control is not calculated, being used as the divisor in the calculation of analgesic power.

The one-way Anova found a significant difference between cumulative total of writhings in the paracetamol group and the ethanolic extract groups at all dosages as compared to the negative control group ( $p < 0.05$ ). Post hoc testing demonstrated that the cumulative totals of writhings differed significantly between the negative control group and the five other intervention groups

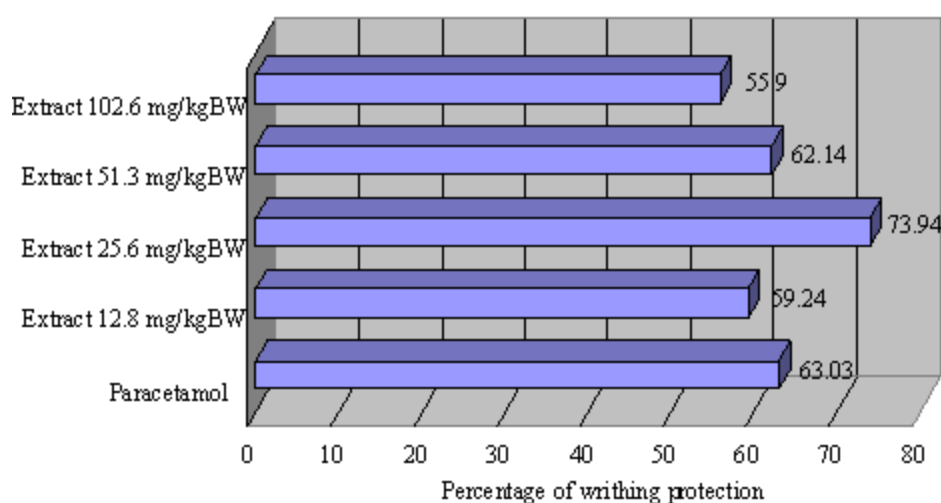


Figure 2. Percentage of writhing protection in intervention groups

( $p < 0.05$ ), but did not differ significantly between the five intervention groups themselves ( $p > 0.05$ ). Calculation of analgesic power with percentage of writhings in the negative control group as divisor showed that paracetamol and ethanolic extract of cassava leaves at all dosages did not differ significantly in analgesic power ( $p > 0.05$ ).

## DISCUSSION

From Table 2 and Figure 2 it is clear that there was an increase in percentage of writhing protection at dosages of 12.8 mg/kgBW and 25.6 mg/kgBW, but a decrease at dosages of 51.3 mg/kgBW and 102.6 mg/kgBW. The graph shows that the analgesic power was highest at the dosage of 25.6 mg/kgBW, whereas at the highest dosage of 102.6 mg/kgBW, the analgesic power was lowest. It is still unclear in the present study why the increase in dosage of the extract was not accompanied by an increase in analgesic power.

The results of the one-way Anova shows that the analgesic power of cassava leaf ethanolic extract at all dosages was significantly different compared with the negative controls ( $p < 0.05$ ), but was not significantly different from that of paracetamol ( $p > 0.05$ ). Therefore it may be concluded that cassava leaf ethanolic extract at dosages of 12.8 mg/kgBW, 25.6 mg/kgBW, 51.3 mg/kgBW, and 102.6 mg/kgBW has an analgesic power equivalent to that of paracetamol at a dosage of 65mg/kgBW. An anti-inflammatory study in rats yielded similar results; the analgesic effect produced by an aqueous cassava leaf extract was significantly higher than that of indomethacin (10 mg/kg, s.c.).<sup>(8)</sup> From the foregoing it can be concluded that *Manihot esculenta* Crantz leaf extracts presumably contain orally safe, topically and orally effective anti-inflammatory and analgesic principles.

Although the present study demonstrated that the cassava plant has an analgesic effect, it

is still unclear what active principles are involved in this effect. One hypothesis is based on the fact that cassava leaves contain flavonoids<sup>(10)</sup> and that nearly all flavonoid compounds possess analgesic activity, due to inhibition of prostaglandin synthesis and consequent decreased stimulation of nociceptors. Flavonoids are thought to interact with the cyclooxygenase system, so as to interfere with arachidonic acid synthesis and inhibit the production of prostaglandins<sup>(21)</sup>.

In addition to the flavonoids, the analgesic effect may also be due to the presence of other antioxidants in the cassava plant, e.g.  $\beta$ -carotenes and vitamin C.<sup>(1,9)</sup> Antioxidants are capable of neutralizing the free radicals released by phagocytes in response to cellular injury, so as to suppress the inflammatory response caused by these radicals, leading to a decreased pain response.<sup>(22)</sup> This idea is supported by the finding that cassava leaves contain anthocyanins, which play an important role in the antioxidant effect<sup>(23)</sup>, while antioxidants in the cassava plant have a demonstrated ability to prevent alcohol-induced hepatotoxicity.<sup>(24)</sup> Other substances to be suspected of playing a role in the analgesic activity are the saponins, as previous studies on different plant species indicate that saponins have analgesic and anti-inflammatory effects, and eliminate pain without affecting cell viability.<sup>(25)</sup>

Which of the possible active compounds in the cassava plant is the most responsible for its analgesic activity is still unclear. The alcoholic solvent used in this study extracts semipolar active compounds, thus it is probable that the analgesic substances are soluble in polar solvents, but to test this suggestion requires further study.

As a preliminary study, the present one has several limitations, among which may be mentioned the simple assessment method of analgesic effect with its high subjectivity level, the relatively narrow dosage range (thus requiring extension), and the limited sample size (five animals for each intervention).



## CONCLUSIONS

Ethanollic extracts of cassava (*Manihot esculenta* Crantz) leaves exert analgesic activity on pain in mice induced by 0.6% acetic acid, but increasing the dosage of the extract beyond a certain level does not result in an increase in analgesic power. Further studies are necessary on the active constituents of cassava leaves that are responsible for their analgesic properties.

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