

Evaluation of the Analgesic Activity of Paracetamol in Male BALB/c Mice Receiving Freshly Expressed Broccoli (*Brassica oleracea* var. *italica*) Juice

Muhammad Baehaqi¹, Ayu Nirmala²

¹ Farmasi Study Program Universitas Pancasakti, Makassar, Indonesia

² Farmasi Study Program Universitas Pancasakti, Makassar, Indonesia

*corresponding email: baehaqihaq45@gmail.com

DOI: -

Received: 06-02-2026

Accepted: 17-05-2026

Published: 20-06-2026

Abstract:

Broccoli contains indole compounds that may induce drug-metabolising enzymes, potentially altering the pharmacological effects of paracetamol. This study aimed to evaluate the effect of freshly expressed broccoli (*Brassica oleracea* var. *italica*) juice on the analgesic activity of paracetamol in male BALB/c mice. The experiment employed a randomised complete block design, with mice randomly assigned to control and treatment groups. Analgesic activity was assessed by recording the cumulative number of writhes over a 60-minute observation period. Mean writhing counts were compared between mice receiving paracetamol alone and those receiving paracetamol following pretreatment with broccoli juice. Statistical analysis was performed using an independent-samples Student's *t*-test at a 95% confidence level. The percentage of analgesic protection was numerically higher in mice pretreated with broccoli juice than in those receiving paracetamol alone, increasing from 54.74% to 71.58%. However, the difference was not statistically significant ($p > 0.05$). These findings indicate that pretreatment with freshly expressed broccoli juice did not significantly alter the analgesic activity of paracetamol under the experimental conditions tested, although a numerical increase in analgesic protection was observed.

Keywords: broccoli juice; paracetamol; analgesic activity; BALB/c mice; writhing test

INTRODUCTION

Paracetamol is a para-aminophenol derivative used as an analgesic and antipyretic. Its inhibition of prostaglandin synthesis in peripheral tissues is relatively weak, and it therefore exhibits no appreciable anti-inflammatory activity. According to Tjay and Rahardja (2002), the analgesic efficacy of paracetamol is comparable to that of aspirin, although its duration of action is slightly shorter. Paracetamol is metabolised primarily in the liver through sulphate and glucuronide conjugation. These processes produce inactive metabolites that are subsequently excreted via the kidneys. A small proportion undergoes metabolism through the hepatic cytochrome P450 enzyme system, predominantly CYP2E1 (Zimmerman and Maddrey, 1995).

Drug metabolism may be influenced by compounds present in food. Vegetables belonging to the Brassicaceae family, including broccoli (**Brassica oleracea* var. *italica**), have been reported to alter the pharmacokinetic parameters of certain drugs. Such alterations may affect the amount of a drug in

the systemic circulation and, consequently, its pharmacological effects. The administration of broccoli in conjunction with medication therefore warrants investigation to clarify the potential interactions involved.

Evidence of such interactions was reported by Sari (1999), who investigated the effects of broccoli pretreatment on the pharmacokinetics of quinidine in rabbits. The study demonstrated reductions in peak plasma concentration and the area under the concentration–time curve, accompanied by significant increases in the volume of distribution and hepatic clearance of quinidine. Changes in pharmacokinetic parameters were also observed following oral quinidine administration. These findings were attributed to a possible effect of broccoli on cytochrome P450 3A4, an enzyme involved in quinidine biotransformation.

Another study indicated that the administration of expressed broccoli juice could influence the pharmacokinetics of tolbutamide in normal rats. Nugroho (2000) reported significant increases in the hepatic clearance and volume of distribution of tolbutamide, accompanied by a reduction in the amount of the drug in the systemic circulation. These findings suggest possible induction of the enzyme systems involved in tolbutamide metabolism. Nevertheless, changes in pharmacokinetic parameters should be distinguished from direct evidence of enzyme induction.

The potential of broccoli to influence drug metabolism is thought to be associated with its indole constituents. Bradfield and Bjeldanes (1991) stated that indole compounds found in **Brassica** species may affect the activity of cytochrome P450-dependent monooxygenases, glutathione S-transferase and epoxide hydrolase. Other publications have also reported effects of cabbage and broccoli consumption on various biotransformation reactions. These include alterations in the metabolism of several compounds in experimental animals, as well as induction of antipyrine and phenacetin biotransformation, paracetamol glucuronidation and glutathione transferase activity in humans (Pantuck et al., 1976; Gibson and Skett, 1986; Beecher, 1994; Cashman et al., 1999). These findings provide a basis for further investigation into the relationship between broccoli administration and pharmacological responses to drugs.

According to Rowland and Tozer (1995), paracetamol, quinidine, tolbutamide, salicylates and phenobarbital are classified as drugs with low hepatic extraction ratios. For these drugs, changes in metabolic enzyme activity may influence both hepatic and total clearance. However, this classification alone does not establish that broccoli administration will diminish the effects of paracetamol. The consequences of any interaction must therefore be examined with reference to the metabolic pathways affected and the resulting changes in pharmacological response.

On this basis, the administration of expressed broccoli juice may influence the analgesic activity of paracetamol by altering its biotransformation. Although previous studies have reported interactions between broccoli and quinidine or tolbutamide, these findings cannot be directly extrapolated to the analgesic

activity of paracetamol. Differences in the drugs investigated, their metabolic pathways and the experimental animals used highlight the need for a specific investigation. Accordingly, this study aims to evaluate the analgesic activity of paracetamol in male BALB/c mice receiving expressed broccoli juice

RESEARCH METHODS

Materials

The materials comprised pharmaceutical-grade paracetamol, analytical-grade glacial acetic acid, broccoli and double-distilled water. Male BALB/c mice aged 2–3 months and weighing 20–30 g were used as experimental animals. Broccoli juice was prepared by processing the broccoli in a blender and expressing the juice without adding water.

Equipment

The equipment included a balance for weighing mice, an oral gavage needle with a 5 mL syringe, a 1 mL syringe for intraperitoneal administration, dropping pipettes, an Eppendorf pipette, volumetric flasks of 10, 50 and 100 mL capacity, Erlenmeyer flasks, beakers, and a mortar and pestle.

Experimental Procedures

The mice were allocated to three experimental groups according to whether they received paracetamol and broccoli juice pretreatment.

Acetic Acid Control Group

Five mice received acetic acid at a dose of 100 mg/kg body weight by intraperitoneal injection. The number of abdominal writhes elicited following administration was recorded.

Paracetamol Group without Broccoli Juice Pretreatment

Six mice received an intraperitoneal injection of acetic acid at 100 mg/kg body weight. Fifteen minutes later, paracetamol was administered orally at 91 mg/kg body weight. The number of abdominal writhes was then recorded.

Paracetamol Group with Broccoli Juice Pretreatment

Five mice received broccoli juice orally at 50 mL/kg body weight per administration. A total of seven administrations were given on a schedule of three administrations per day. Five hours after the final administration of broccoli juice, the mice received an intraperitoneal injection of acetic acid at 100 mg/kg body weight. Paracetamol was administered orally at 91 mg/kg body weight 15 minutes later, and the number of abdominal writhes was recorded.

Assessment of Analgesic Activity

Analgesic activity was calculated from the recorded writhing counts using the following equation:

$$\text{Analgesic activity (\%)} = \frac{\overline{W}_c - \overline{W}_t}{\overline{W}_c} \times 100\%$$

Keterangan:

$\overline{W}_c$ = rerata jumlah geliat kelompok kontrol;

$\overline{W}_t$ = rerata jumlah geliat kelompok perlakuan

Analgesic activity was expressed as the percentage reduction in the mean writhing count relative to the acetic acid control group.

Statistical Analysis

Mean writhing counts were compared between the acetic acid control group and the paracetamol groups, with and without broccoli juice pretreatment, using an unpaired Student's t-test. Statistical significance was assessed at the 5% level, corresponding to a confidence level of 95%

RESULTS AND DISCUSSION

Analgesic activity was evaluated using the acetic acid-induced writhing test, in which intraperitoneal administration of acetic acid produces a chemical nociceptive stimulus. The resulting response is manifested as abdominal writhing. The behavioural criteria used to identify this response included extension of the forelimbs forwards and the hindlimbs backwards, accompanied by the abdomen pressing against the cage floor. The initial response was characterised by at least one or two writhing episodes within five minutes of acetic acid administration (Vogel, 2002).

Acetic acid was administered at 100 mg/kg body weight, and writhing episodes were recorded over a 60-minute observation period. The control group exhibited a mean cumulative writhing count of 95 ± 10 , expressed as the mean $\pm$ standard error of the mean (SEM), with individual counts ranging from 70 to 124. Analgesic activity was calculated as the percentage reduction in the mean writhing count relative to the acetic acid control group. The results are presented in Table 1.

Table 1.

Analgesic activity of paracetamol with and without broccoli juice pretreatment in male BALB/c mice

Treatment	Cumulative writhing count (mean $\pm$ SEM)	Analgesic activity (%)
Acetic acid control	95 ± 10	0.00
Paracetamol	43 ± 11	54.74
Paracetamol with broccoli juice pretreatment	27 ± 7	71.58

To assess the analgesic activity of paracetamol alone, male BALB/c mice received an intraperitoneal injection of acetic acid at 100 mg/kg body weight, followed 15 minutes later by oral paracetamol at 91 mg/kg body weight. Paracetamol significantly reduced the mean writhing count compared with the acetic acid control group, from 95 ± 10 to 43 ± 11 ($p < 0.05$). Individual counts in the paracetamol group ranged from 3 to 63. This reduction corresponded to an analgesic activity of 54.74%. The mean writhing counts at successive observation intervals are presented in Figure 1.

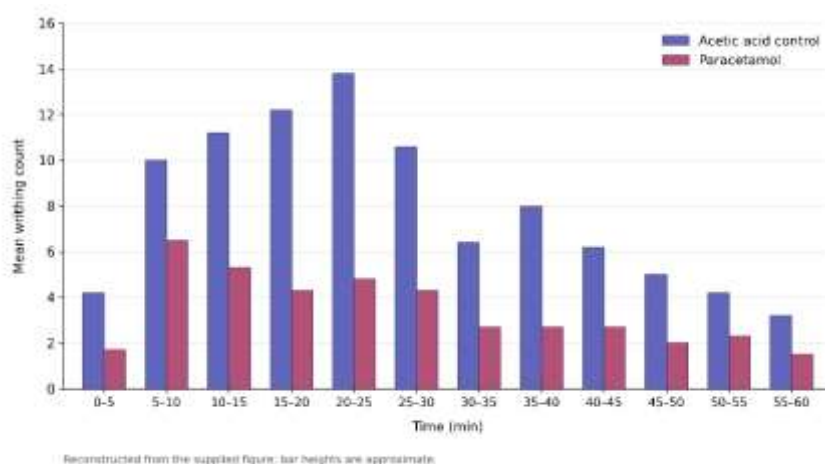


Figure 1

Mean writhing counts at five-minute observation intervals in male BALB/c mice receiving acetic acid (100 mg/kg, intraperitoneally), with or without subsequent oral paracetamol (91 mg/kg).

As illustrated in Figure 1, the paracetamol-treated group exhibited lower mean writhing counts than the acetic acid control group throughout the observation period. In the control group, the mean writhing count generally increased during the early intervals, reaching its highest value at 20–25 minutes before declining overall towards the end of the observation period. By comparison, the paracetamol-treated group showed its highest mean count at 5–10 minutes, followed by a generally decreasing pattern with minor fluctuations. These observations are consistent with the lower cumulative writhing count recorded following paracetamol administration. However, the figure alone does not establish statistical significance at individual observation intervals.

The effect of broccoli juice pretreatment was assessed following oral administration at 50 mL/kg body weight per administration. Seven administrations were given over three consecutive days: three administrations daily for the first two days and one on the third day. Five hours after the final administration, the mice received acetic acid intraperitoneally at 100 mg/kg body weight, followed 15 minutes later by oral paracetamol at 91 mg/kg body weight. Writhing episodes were subsequently recorded for 60 minutes.

The broccoli-pretreated group exhibited a mean cumulative writhing count of 27 ± 7 , with individual counts ranging from 12 to 43. Relative to the acetic acid control group, this represented an analgesic activity of 71.58%. Compared with paracetamol alone, broccoli pretreatment was associated with a numerically lower mean writhing count, decreasing from 43 ± 11 to 27 ± 7 . Correspondingly, calculated analgesic activity increased from 54.74% to 71.58%, a difference of 16.84 percentage points. However, the difference between the two paracetamol groups was not statistically significant ($p > 0.05$).

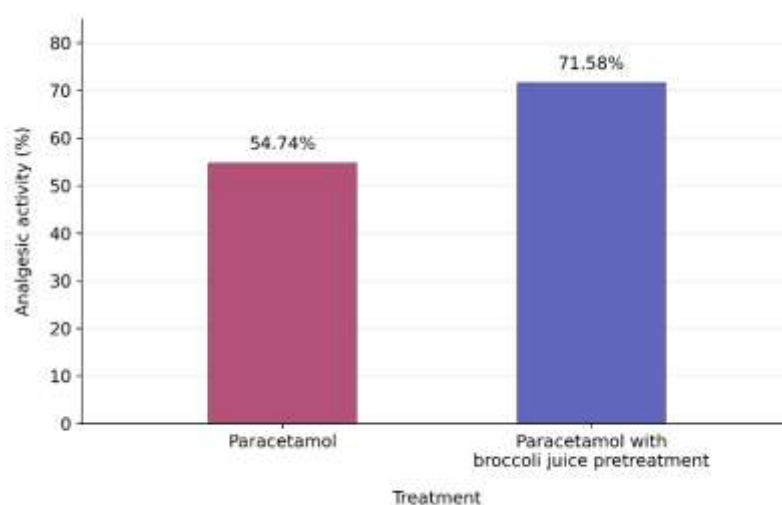


Figure 2.

Analgesic activity of oral paracetamol (91 mg/kg body weight) in male BALB/c mice with and without broccoli juice pretreatment (50 mL/kg body weight per oral administration; seven administrations).

These findings indicate that broccoli juice pretreatment did not produce a statistically significant change in the analgesic activity of paracetamol under the experimental conditions employed. Although the numerical results suggested greater inhibition of writhing following pretreatment, they do not establish an enhancement of the analgesic effect. Equally, the absence of statistical significance should not be interpreted as evidence that the treatments are equivalent or that an interaction is absent.

One possible explanation concerns the metabolic pathways involved in paracetamol biotransformation. Zimmerman and Maddrey (1995) describe phase II conjugation as the predominant route, with a smaller proportion undergoing oxidative metabolism involving CYP2E1. Ritschel (1992) similarly identifies sulphate and glucuronic acid conjugation as important pathways in paracetamol metabolism. The effects on cytochrome P450 3A discussed in relation to broccoli may therefore not translate directly into a measurable alteration in paracetamol analgesia. However, this remains a proposed explanation, as the present study did not directly measure enzyme activity, paracetamol concentrations or metabolite formation.

Overall, broccoli juice pretreatment was associated with a numerical increase in calculated analgesic activity, but the difference from paracetamol alone was not statistically significant. The findings therefore provide insufficient evidence that the pretreatment regimen altered the analgesic response to paracetamol in male BALB/c mice. They also do not establish whether broccoli induced or inhibited phase I or phase II metabolic enzymes

CONCLUSION AND RECOMMENDATIONS

Conclusion

Broccoli juice pretreatment was associated with a numerical increase in the analgesic activity of paracetamol in male BALB/c mice, from 54.74% to 71.58%. However, the difference was not statistically significant ($p > 0.05$). These findings therefore provide insufficient evidence that broccoli juice pretreatment enhances the analgesic activity of paracetamol under the experimental conditions investigated.

Recommendations

1. Further research should investigate whether broccoli constituents influence the distribution of paracetamol or interact with the molecular pathways involved in its analgesic action. Direct assessment of these processes would help clarify the mechanisms underlying any potential interaction.
2. Future studies should examine potential interactions between Brassicaceae vegetables and other commonly used medicines, including drugs with low hepatic extraction ratios. The selection of these medicines should take account of their principal metabolic and elimination pathways to provide a clearer understanding of the factors governing such interactions.
3. Further investigations should evaluate different doses and durations of broccoli juice pretreatment, with appropriate consideration of tolerability and animal welfare. Direct measurements of paracetamol and its metabolites, together with assessments of phase II metabolic activity, would help determine whether broccoli juice influences paracetamol biotransformation.

REFERENCES

- Beecher, C. W. W. (1994). Cancer prevention properties of varieties of *Brassica oleracea*: A review. *American Journal of Clinical Nutrition*, 59(Suppl.), 1165S–1170S.
- Bradfield, C. A., & Bjeldanes, L. F. (1991). Modification of carcinogen metabolism by indolylic autolysis products of *Brassica oleracea*. *Advances in Experimental Medicine and Biology*, 289, 153–163.
- Cashman, J. R., Xiong, Y., Lin, J., Verhagen, H., van Poppel, G., van Bladeren, P. J., Larsen-Su, S., & Williams, D. S. (1999). In vitro and in vivo inhibition of human flavin-containing monooxygenase form 3 (FMO3) in the presence of dietary indoles. *Biochemical Pharmacology*, 58, 1047–1055.
- Gibson, G. G., & Skett, P. (1986). *Introduction to drug metabolism*. London: Chapman and Hall.
- Nugroho, A. E. (2000). *Pengaruh praperlakuan brokoli (Brassica oleraceae L.) terhadap terhadap farmakokinetika tolbutamid pada tikus putih jantan* [Laporan penelitian]. Yogyakarta: Lembaga Penelitian Universitas Gadjah Mada.
- Pantuck, E. J., Hsio, K. C., Loub, W. D., Wattenberg, L. W., Kuntzman, R., & Conney, A. H. (1976). Stimulatory effect of vegetables on intestinal drug metabolism in the rat. *Journal of Pharmacology and Experimental Therapeutics*, 198, 278–283.

- Ritschel, W. A. (1992). *Handbook of basic pharmacokinetics* (4th ed.). Hamilton: Drug Intelligence Publications.
- Rowland, M., & Tozer, T. N. (1995). *Clinical pharmacokinetics: Concepts and applications* (3rd ed.). Philadelphia: Lippincott Williams & Wilkins.
- Sari, I. P. (1999). *Antaraksi farmakokinetika brokoli (Brassica oleraceae L. vabrotrytis L.)-kinidin: Kajian terhadap akibat dan perantara antaraksi*. Yogyakarta: Fakultas Farmasi, Universitas Gadjah Mada.
- Tjay, H. T., & Rahardja, K. (2002). *Obat-obat penting* (Edisi ke-5). Jakarta: Direktorat Jenderal Pengawasan Obat dan Makanan, Departemen Kesehatan Republik Indonesia.
- Vogel, H. G. (2002). *Drug discovery and evaluation: Pharmacological assays*. Springer.
- Zimmerman, H. J., & Maddrey, W. C. (1995). Acetaminophen (paracetamol) hepatotoxicity with regular intake of alcohol: Analysis of instances of therapeutic misadventure. *Hepatology*, 22(3), 767-773