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The Chemical Profile and Potency of *Albertisia papuana* Leaves in Suppressing Withdrawal Symptoms of Morphine Addiction in Mice

Hajrah Hajrah[1*], Niken Indriyanti[1], Mukti Priastomo[1], Erwin Samsul[1], Wisnu Cahyo Prabowo[2]
[1]Department of Pharmacology, Faculty of Pharmacy, Mulawarman University,
Samarinda, East Borneo, Indonesia
[2]Department of Biology, Faculty of Pharmacy, Mulawarman University,
Samarinda, East Borneo, Indonesia
*Correspondence author (e-mail: hajrahsuhardi@gmail.com)

Opiate addiction is a very troublesome issue, with the number of cases increasing every year. Rehabilitation methods that are currently available are often unsatisfactory as the drugs used for the detoxification process can inflict adverse effects such as cravings and respiratory depression. *Albertisia papuana* leaves, commonly called *mekai* by the Dayak tribe, are often used by these people to enhance the taste of food. This plant has antidotal potential and affects the nervous system. However, research on this plant is still scarce, thus its effectiveness in overcoming drug dependence is not yet proven. We attempted to explore this plant by analysing its chemical content using LC-MS/MS and assessing its potential as a withdrawal symptom suppressor in a model of narcotic dependence by observing addictive behaviour in mice. The chemical profile of *A. papuana* included $C_7H_{16}NO_3$, $C_{20}H_{26}NO_3$, $C_{20}H_{24}NO_4$, $C_{19}H_{37}N_4O_3$, $C_{21}H_{22}NO_4$, and $C_{47}H_{50}N_9O_{12}$. The observation of withdrawal behaviour was done using psychomotor tests, curiosity and coordination tests, as well as light and ear nerve sensitivity tests. The results showed values that were close to the control group, with consecutive values of 12.38 ± 5.25 seconds, 3.75 ± 4.35 seconds, 4.00 ± 3.74 seconds, 60.25 ± 51.08 seconds and 139.50 ± 61.70 seconds for mice that were given *A. papuana* leaf extracts. In all tests, *A. papuana* significantly reduced withdrawal effects. This data may be used for further research on the use of *A. papuana* leaves in rehabilitation treatments for morphine addiction.

Key words: *Albertisia papuana*; Withdrawal Symptoms; Mekai

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Drug addiction is a global problem. According to the latest World Drug Report released in 2021 by the United Nations Office on Drugs and Crime [1], in 2020 approximately 275 million people used drugs and over 36 million suffered from drug use disorders. Addiction is defined as a chronic, relapsing disorder characterized by compulsive drug seeking, continued use despite harmful consequences, and long-lasting changes in the brain, and is considered both a complex brain disorder and a mental illness [2]. It is not an easy feat to end addiction immediately due to the emergence of withdrawal symptoms during the process. Withdrawal symptoms occur when an individual attempts to stop the use of addictive substances, and this leads to a cycle of dependency which makes it harder for them to stop [3].

A common approach in morphine addiction rehabilitation is detoxification followed by psychosocial therapy to prevent relapse [4]. FDA-approved medication for the treatment of opioid dependence include methadone, buprenorphine and naltrexone [5]. Methadone works as an opioid agonist in the brain to reduce withdrawal symptoms and drug cravings. Methadone is a full-fledged opioid agonist, and hence has the potential to be abused and can cause dependence in patients [5]. Buprenorphine is a partial opioid agonist that works by partially occupying opioid receptors in the brain, but due to the low rate of opioid abstinence, it is generally used as a transitional therapy before methadone or naltrexone is prescribed [5]. Naltrexone is an opioid antagonist that works by blocking the activation of opioid receptors, thus reversing the effects of opioids. However it can also cause depression in the respiratory tract and cardiovascular complications, therefore its use needs to be closely monitored [6].

The rehabilitation methods currently available are considered unsatisfactory as the drugs used in the detoxification process inflict adverse effects such as cravings and respiratory depression [5]. A new approach to treat narcotic addiction and withdrawal symptoms is by using glutamatergic substrates.

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Drug abuse over a long period of time seems to modulate glutamatergic transmission, resulting in long-term neuroplastic changes in the brain that may contribute to drug-seeking behaviour and drug-related memory [7]. Glutamatergic substrates such as N-acetylcysteine, lamotrigine, topiramate, memetine and gabapentin may suppress glutamatergic transmission thereby reducing relapse-like behaviour or potentiate glutamatergic transmission which may facilitate elimination of drug-seeking behaviour [8].

Mekai or *Albertisia papuana* is a plant of the Menispermaceae family used by the Dayak tribe in Kalimantan as a spice, antidote, as well as a herbal medicine to treat cancer. Its anticancer activity has been studied *in vitro* [9-10]. Research on this plant has been limited to the content of free glutamic acid found in the leaves, which is around 101.83 mg/g [11]. Therefore, it is necessary to conduct further research to determine the chemical compounds present in this plant and to assess its potential as a glutamatergic substrate that can be used to suppress the withdrawal symptoms of drug addiction.

EXPERIMENTAL

Animals

Male mice of strain DDY (n = 12, Universitas Gadjah Mada Yogyakarta) at 12-16 weeks of age were placed in cages with a 12-hour light/dark cycle with free access to food and drink facilities. Mice were acclimatized for one week before testing and weighed periodically.

Drugs

A. papuana leaves were obtained from the Malinau district of North Kalimantan and analysis of the plants was carried out at the Faculty of Forestry, Mulawarman University. Morphine was purchased from Kimia Farma in the form of MST tablets and its use was submitted to the BNN (National Anti-narcotics Agency) Samarinda. *Mekai* leaf extracts and morphine were in the form of a suspension using a Na-CMC carrier to facilitate oral administration of the drug.

Profiling Compounds of *A. papuana* Leaf Extract

Dried *A. papuana* leaves were macerated and extracted with 96% ethanol. The *A. papuana* leaf extracts were analyzed using the HPLC-qTOF-MS tandem system AB Sciex 4000 at the Angler Biochemlab laboratory in Surabaya.

Effect of *A. papuana* Leaves on Morphine-induced Withdrawal Symptoms in Mice

Mice were divided into 3 groups by a randomization method. Group 1 was a control group that was not given any morphine or treatment. Group 2 was given *A. papuana* leaf extracts at a dose of 4 mg/kg BW, while group 3 was given a placebo. Each group consisted of 4 mice. Before treatment, mice in groups 2 and 3 were conditioned to experience withdrawal symptoms with the morphine rechallenge-dechallenge induction method which lasted 21 days. In the first week, the mice were dosed with morphine at 0.026 mg/20 grams body weight, then the dose was doubled at week 2 to 0.052 mg/20 grams body weight and 0.104 mg/20 mg body weight at week 3. On the 7th and last day, addiction behaviours such as ataxia, aggressiveness, piloerection, grip strength, tail erection, tail movement, circular motion, skin coloration of ears and tail, alertness, catalepsy, convulsions, muscle wrinkling, lacrimation, miosis, mydriasis, fear, writhing, breathing, nystagmus, tremor, paralysis, reflexes, salivation, and urination were observed. Mice showing no signs of addiction were excluded from the study. Ethanol extracts of *A. papuana* leaves and a placebo were given for 14 days and on the last day of the study, the mice were observed using psychomotor tests, ear nerve sensitivity tests, light sensitivity tests, curiosity tests, and coordination behaviour tests [12-20].

RESULTS

79 g of *A. papuana* leaves were macerated and extracted with 96% ethanol. The weight of the extract obtained was 4.6 grams, giving a yield of 5.823%. The HPLC chromatogram profile and mass spectrum data of *A. papuana* leaf extracts using HPLC-qTOF-MS, showed 5 peaks with retention times of 1.24, 3.89, 4.18, 5.44 and 8.53 min (Figure 1).

The chromatogram and mass spectrum data showed that 5 compounds (Table 1) were detected in the *A. papuana* leaf extracts. At a retention time of 1.24 min, a compound was detected with the molecular formula $C_7H_{16}NO_3$ (molecular weight 162.1125), which may be 3-morpholino-1,2-propanediol. At 3.89 min, another compound was detected, with the molecular formula $C_{20}H_{26}NO_{31}^+$ (molecular weight 328,190) or $C_{20}H_{24}NO_{41}^+$ (molecular weight 342.1700). At 4.18 min, $C_{19}H_{37}N_4O_{31}^+$ (369.2860) or $C_{20}H_{24}NO_{41}^+$ (342.1700) was also found. At 5.44 min, the compound $C_{21}H_{22}NO_{41}^+$ was detected, with a molecular weight of 352.1543. Lastly at 8.53 min, $C_{47}H_{50}N_9O_{121}^+$ was detected, with a molecular weight of 932.3573. There were 4 more compounds present which have not yet been identified. Further analysis is necessary to determine the names and classes of these compounds.

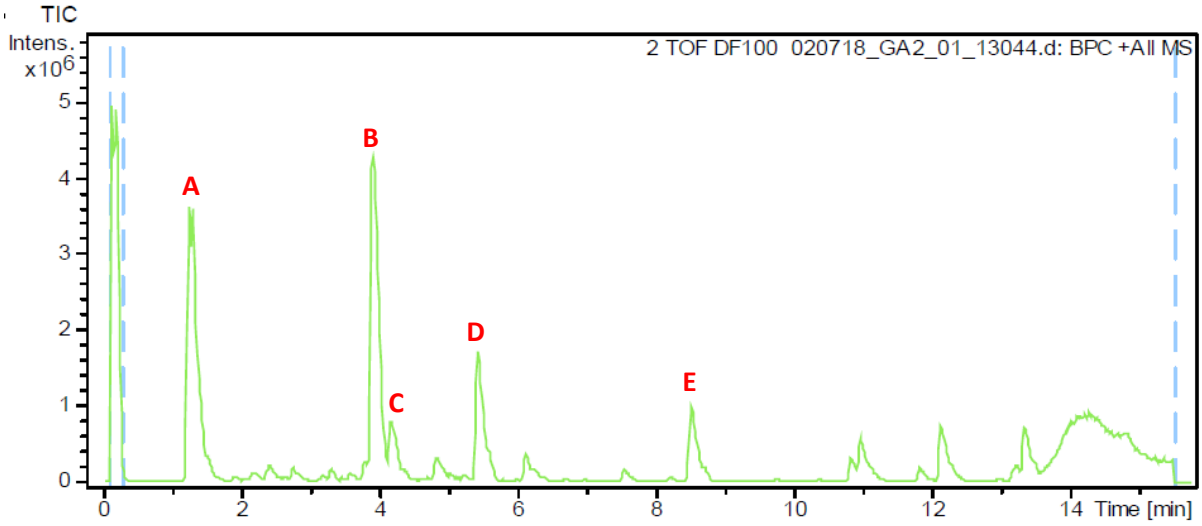

Figure 1. Chromatogram of *A. papuana* leaves extract. Peak (A) had a retention time of 1.24, (B) a retention time of 3.89, (C) a retention time of 4.18, (D) a retention time of 5.44 and (E) a retention time of 8.53.

Table 1. HPLC-qTOF-MS data for the ethanol extract of *A. papuana* leaves

No	Retention Time	Precursor Ion Ion formula	m/z	err (mDa)	m Sigma	Possibility Name
1	1.24	$C_7H_{16}NO_3^{1+}$	162.1125	0.6	2.9	3-Morpholino-1,2-propanediol
2	3.89	$C_{20}H_{26}NO_3^{1+}$	328.1907	0.7	9.9	-
		$C_{20}H_{24}NO_4^{1+}$	342.1700	0.7	10.6	-
3	4.18	$C_{19}H_{37}N_4O_3^{1+}$	369.2860	0.0	9.4	-
		$C_{20}H_{24}NO_4^{1+}$	342.1700	-1.1	3.5	-
4	5.44	$C_{21}H_{22}NO_4^{1+}$	352.1543	0.8	10.5	-
5	8.53	$C_{47}H_{50}N_9O_{12}^{1+}$	932.3573	1.0	18.1	-

Table 2. Assessment of the neurologic effects of *A. papuana* extract on the behaviour of morphine-addicted mice

Group	Psychomotoric test by swimming (mean ± SD sec)	Ear nerves sensitivity test (mean ± SD sec)	Curiousity test (mean ± SD sec)	Coordination behavior by crossing a line (mean ± SD sec)	Light sensitivity test (mean ± SD sec)
Normal	14.67±0.58	220.67±15.98	3.00±1.73	6.00±3.00	66.00±33.87
Addicted mice treated with *A. papuana* leaf extracts	12.38±5.25	139.50±61.70	3.75±4.35	4.00±3.74	60.25±51.08
Addicted mice treated with placebo	10.73±7.39	304.00±79.19	8.00±8.49	2.00±0.00	201.00±234.76

n= 4 mice per group

The potential of *A. papuana* leaf extract in suppressing withdrawal symptoms in morphine-addicted mice was assessed from the changes in the behaviour of mice treated with 4 mg/kg BW of the extract compared to the mice that were given placebos and those in the control group. The behaviour assessed was related to the psychomotor skills, nerve and light sensitivity, curiosity and coordination of the mice. The results are shown in Table 2.

Psychomotor abilities were observed using a swimming test. Addicted mice treated with *A. papuana* leaf extracts showed a swimming time of 12.38 seconds, which was close to that for control mice (14.67 seconds). Addicted mice treated with a placebo were able to swim for 10.73 seconds. The ear nerve sensitivity test showed that the time required for addicted mice treated with the leaf extracts to respond to sound was 139.50 seconds, which was much faster than that for normal mice (220.67 seconds), while for addicted mice treated with a placebo, it was 304.00 seconds. Similar results were found with the light sensitivity test, where addicted mice treated with *A. papuana* leaf extracts were able to respond to light within 60.25 seconds compared to the addicted mice treated with a placebo, that took 201.00 seconds.

The curiousity test value for addicted mice treated with *A. papuana* leaf extracts was 3.75 seconds while for normal mice and addicted mice treated with a placebo, the values were 3.00 and 8.00 seconds, respectively. The coordination behaviour test measured the ability of a mouse to cross a traction wire, and the results showed that normal mice were able to hold on for 6.00 seconds, while addicted mice treated with *A. papuana* leaf extracts could hold on for 4.00 seconds and addicted mice treated with a placebo, for 2.00 seconds.

DISCUSSION

The *mekai* plant used in this study was identified by the Dendrology and Ecology Laboratory, Faculty of Forestry, Mulawarman University, and belongs to the species *A. papuana* Becc. synonymous with *A. papuana* var Buncana Boerl and *A. papuana* var buruensis Boerl.

A. papuana leaves contain glutamic acid which creates the umami taste in food [22] and acts as a glutamatergic substrate to modulate glutamate transmission in the brain, which can suppress the withdrawal symptoms of morphine addiction. Another constituent of *A. papuana* is 3,4-dihydro-6,7-dimethoxy isoquinoline 2-oxide, which is an isoquinoline alkaloid [21] that may contribute as an anti-addictive in this test [22].

Withdrawal symptoms are a series of physical and psychological symptoms caused by drug withdrawal. Parameters that can be analysed to determine the severity of withdrawal symptoms include behaviours that arise due to stimulation or depression of the nervous system such as feelings of pain, anxiety or agitation, muscle cramp, restlessness and depression.

Withdrawal behaviour was observed by using psychomotor tests, curiosity and coordination tests, light sensitivity tests and ear nerve sensitivity tests. The results are shown in Table 2. The psychomotor test using swimming showed that there was an increase in swimming time for mice given the *A. papuana* leaf extracts compared to the placebo group, and the value was close to the swimming test value for the control group. This indicates that there was an increase in performance in the treated mice, thus it can indirectly be concluded that there was an improvement in the symptoms of depression experienced by these experimental animals.

The improvement of symptoms due to nervous system depression could be observed in the test results for hearing and vision sensitivity in the mice, where there was an increase in the response time of the treated mice to stimuli when compared to the group given a placebo. This shows that there was an increase in awareness of the mice, marked by the shorter time it took for them to respond to sound and light.

The curiosity test observed excessive curiosity and uneasiness that indicated anxiety. In Table 2, it is clear that the mice treated with *A. papuana* extracts showed a decrease in curiosity compared to the placebo group and this value was close to the control group. This indicates that the *A. papuana* leaf extract was able to reduce the aggressive action of mice due to anxiety. The coordination behaviour test showed that mice given *A. papuana* leaf extracts experienced an increase in the amount of time they were able to hold on to the traction wire. This data reflects the fact that muscle and nerve coordination were generally better in the treated mice compared to mice given a placebo, and were similar to normal mice.

Due to instrumental limitations, the observation of addictive behaviour was done under less than ideal conditions. Observations were limited to changes in psychomotor behaviour of subjects before and after treatment. Therefore, it is necessary to carry out further testing with more specific observation parameters such as impaired-response inhibition salience attribution (I-RISA), drug self-administration, multistimuli behaviour and place conditioning, or by looking at morphine deposits in the prefrontal cortex area of the brain using an immunohistochemical method. In addition, this study did not use a positive control comparison, thus further research is needed to assess the ability of this plant to overcome withdrawal symptoms in comparison with standard drugs.

CONCLUSION

A. papuana (*mekai*) leaves were found to contain $C_7H_{16}NO_3$, $C_{20}H_{26}NO_3$, $C_{20}H_{24}NO_4$, $C_{19}H_{37}N_4O_3$, $C_{21}H_{22}NO_4$ and $C_{47}H_{50}N_9O_{12}$. Ethanol extracts of these leaves were found to have potential as a suppressor of withdrawal symptoms in morphine-addicted mice at a dose of 4 mg/kg BW. Further research needs to be conducted to identify other compounds present in *A. papuana* leaves and to assess their use in the rehabilitation of morphine dependence.

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