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## Antinociceptive activity of methanol extract of *Chlorophytum alismifolium* tubers in murine model of pain: Possible involvement of $\alpha_2$ -adrenergic receptor and $K_{ATP}$ channels

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### ABSTRACT

The tubers of *Chlorophytum alismifolium* are used in Nigerian Herbal Medicine for the management of diabetes mellitus, painful and inflammatory conditions. The antinociceptive activity has been validated but the mechanism of this activity is yet to be explored. This study therefore, aimed to investigate the probable mechanism(s) of the antinociceptive activity of *C. alismifolium* tubers using experimental animal model of pain. HPLC and GC-MS analyses were carried out on the extract. Antinociceptive activity was investigated using acetic acid-induced writhing response test in mice. Three groups of mice were orally administered distilled water (10 ml/kg), *C. alismifolium* (400 mg/kg) and morphine (10 mg/kg) 60 min before administration of acetic acid and the resulting writhing were counted for 10 min. To establish the probable mechanism(s) of action of *C. alismifolium*, separate groups of animals were pre-treated intraperitoneally with naloxone (2 mg/kg), prazosin (1 mg/kg), yohimbine (1 mg/kg), propranolol (20 mg/kg) and glibenclamide (5 mg/kg) 15 min before *C. alismifolium* administration. HPLC chromatogram of the extract revealed seventeen characteristic peaks with retention times ranging between 2.1 and 7.4 min. Administration of *C. alismifolium* significantly ( $p < 0.01$ ) reduced the mean number of writhes compared to control group. Pretreatment with yohimbine and glibenclamide significantly ( $p < 0.05$  and  $p < 0.01$  respectively) reduced the antinociceptive activity of extract-alone treated group. However, pretreatment with prazosin, naloxone and propranolol showed no effect on its analgesic activity. The findings from this research revealed the possible involvement of  $\alpha_2$ -adrenergic receptor and  $K_{ATP}$  channels in the antinociceptive activity of *Chlorophytum alismifolium* tuber extract.

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### 1. Introduction

Herbal medicines have been widely used in the treatment of many diseases because of their therapeutic benefits<sup>1</sup> and they are sources of lead compounds, some of which are very useful synthetic class of modern drugs.<sup>2</sup> Medicinal herbs have been used as a form

of therapy for relieve of pain throughout history<sup>3</sup> with little or no toxicity; and the most important analgesic prototypes (e.g. acetyl salicylic acid and morphine) were originally derived from plant sources. It is widely known that conventional analgesics such as opiates and non-steroidal anti-inflammatory drugs are associated with serious side effects.<sup>4</sup> These drawbacks can be bypassed or minimized by substituting them with effective and safer medicinal plants.<sup>5</sup> One of such plants that has gained wide patronage in the management of pain is *Chlorophytum alismifolium*.

*Chlorophytum alismifolium* (Family: Liliaceae) is a short stem herb with tuberous root stocks and white flowers found around stony sites in forest streams.<sup>6</sup> It is commonly known as Alimsa-

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Ground lilly, and locally as *Rogon makwarwa* (Hausa) and *Cigorodi* (Fulfulde). The tubers are widely used traditionally for the management of pain and inflammatory conditions.<sup>7</sup>

According to Bellik et al.,<sup>8</sup> a number of medicinal plants with analgesic activity have been documented, but limited information is available with respect to their mechanisms of antinociceptive activity. The antinociceptive and anti-inflammatory activities of the tubers of *C. alismifolium* have previously been reported.<sup>7</sup> Therefore, the aim of this study was to investigate the probable mechanism(s) of antinociceptive activity of the standardized extract of *C. alismifolium* tubers through the involvement of opioidergic, adrenergic and potassium ATP pathways using an experimental animal model of pain. The dose of methanol extract of *C. alismifolium* with the best antinociceptive activity (400 mg/kg) from previous studies<sup>7</sup> was used for the present study.

## 2. Materials and methods

### 2.1. Animals

Swiss albino mice (18–24 g) of either sex were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The animals were maintained under standard environmental conditions and fed with standard pelletized rodent diet and water *ad libitum*. The experimental protocols were approved by Ahmadu Bello University Animal Ethics Committee (Protocol number: DAC/IW-OT/212-15) and the animals were handled according to the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised, 1996).

### 2.2. Drugs and chemicals

Naloxone, Prazosin, Glibenclamide, Yohimbine and Propranolol (Abcam Biochemicals Plc, Cambridge, UK), Morphine sulphate (Martindale Pharmaceuticals, U.K), Glacial acetic acid (May and Baker Limited, England).

### 2.3. Plant material

The tubers of *C. alismifolium* were collected in July 2014 from *Tilden Fulani* River in Toro LGA, Bauchi State, Nigeria. The botanical identification and authentication was done by Mallam Musa Muhammed of the Herbarium Unit of the Department of Botany, Ahmadu Bello University, Zaria, Nigeria. A voucher number of 6785 was obtained and a voucher specimen was kept in the Herbarium for future reference.

### 2.4. Preparation of *Chlorophytum alismifolium* extract (CAE)

The tubers were washed and chopped into smaller sizes and then air-dried under shade for three weeks until constant weight was attained. The dried plant was then crushed into fine powder using pestle and mortar. The powdered plant (1 kg) was extracted with 2.5 L of 90% v/v aqueous methanol (90% methanol: 10% water) for 72 h using the soxhlet apparatus. The extract was concentrated to dryness on a water bath set at 50 °C and was stored in a desiccator until required for the main experiment.

### 2.5. Phytochemical screening

Standard phytochemical screening tests were employed in screening CAE.<sup>9,10</sup> The extract was screened for the presence or absence of phytochemicals including alkaloids, flavonoids,

saponins, glycosides, cardiac glycosides, tannins, anthraquinones and triterpenes.

### 2.6. Chromatographic analyses

#### 2.6.1. High performance liquid chromatography (HPLC) analysis

The procedure was carried out using HPLC1260 VWD VL (Agilent Technologies, UK) on a Techsphere C<sub>18</sub> column (250 mm × 4.6 mm, 5 μ particle size) by isocratic elution with methanol/water 80:20 as the mobile phase. The flow rate was set at 1 ml/min, injection volume of 10 μL of methanol solution of *C. alismifolium* extract and the detection was at 232 nm, under an ambient column temperature. The total run time for the analysis was 8 min.

#### 2.6.2. Gas chromatography-mass spectroscopy analysis (GC-MS)

GC-MS analysis was performed using an Agilent 7890B GC system, 5977A mass spectrum detector (MSD) (Agilent Technologies, USA). The chromatography was performed on a HP-5 MS capillary column (30 m × 250 μm × 0.25 μm). The carrier gas used was high purity helium and the constant flow rate of the helium was 3.6839 mL/min. Split injection ratio was 5:1. The temperature of the GC started at 50 °C for 1 min, raised to 200 °C at a rate of 3 °C/min and then raised to 300 °C at 3 °C/min for 15 min and then held at 325 °C (1 min). MS program scanned quality range of 30amu - 600amu, ionization voltage of 70eV, ionization current of 150 μA (EI). The ion source and the quadrupole temperature were set at 230 °C and 150 °C respectively. Compounds in the extract were identified on the basis of standards, isolation and structural determination in our laboratory, NIST 14.L database.<sup>11</sup>

### 2.7. Acute toxicity study

The method described by Lorke<sup>12</sup> was employed in the determination of the oral median lethal dose (LD<sub>50</sub>) in mice. The test was in two phases; in phase one, three groups of animals (n = 3) were administered widely differing doses of the extract (10, 100 and 1000 mg/kg) and were observed for signs of toxicity and mortality for 24 h. In the second phase, 3 animals were administered 1600, 2900 and 5000 mg/kg of the extract and then observed for signs of toxicity and mortality for 24 h. The LD<sub>50</sub> was subsequently estimated.

### 2.8. Antinociceptive studies

#### 2.8.1. Acetic acid-induced writhing in mice

The method described by Koster et al.<sup>13</sup> was employed. A total of 18 mice were divided into three groups of six mice each. Group I was administered distilled water (10 ml/kg), groups II and III received 400 mg/kg of the extract and 10 mg/kg of morphine respectively, all through the oral route (*p.o.*). Sixty min. post treatment, all the mice received 10 ml/kg of 0.6% v/v acetic acid intraperitoneally. Five minutes after acetic acid injection, the mice were placed in individual observation cages and the number of abdominal writhing was recorded for each mouse for a period of 10 min. A decrease in the number of writhes as compared to the control group mice was considered as evidence of antinociception and expressed as percentage inhibition of writhes using the relationship below:

$$\% \text{ Inhibition} = \frac{\text{Mean number of writhes (control)} - \text{Mean number of writhes (test)}}{\text{Mean number writhes (control)}} \times 100$$

### 2.9. Evaluation of the pharmacological mechanisms of antinociceptive action of CAE

To investigate the possible mechanisms involved in the antinociceptive effect of CAE, randomly selected mice ( $n = 6$ ) were pretreated with the following agents: naloxone (2 mg/kg, *i.p.*), a competitive opioid antagonist; Prazosin (1 mg/kg, *i.p.*), a selective  $\alpha_1$ -adrenoceptor blocking agent, Yohimbine (1 mg/kg, *i.p.*), a pre-synaptic  $\alpha_2$ -adrenoceptor blocking agent; Propranolol (20 mg/kg, *i.p.*), a non-selective  $\beta$ -adrenoceptor blocking agent and Glibenclamide (5 mg/kg, *i.p.*) an inhibitor of ATP-sensitive potassium channels ( $K_{ATP}$ ); 15 min before oral administration of CAE (400 mg/kg). In two separate groups of mice ( $n = 6$ ), the effects of the vehicle (distilled water, 10 ml/kg) and CAE (400 mg/kg) were assessed. Sixty minutes post treatment, 10 ml/kg of 0.6% $^v/v$  acetic acid was administered intraperitoneally to mice in all groups to induce writhing. Five minutes after the acetic acid injection, the mice were placed in individual observation cages and the number of abdominal writhes were recorded for each mouse for a period of 10 min.<sup>14,15</sup>

### 2.10. Statistical analysis

The data obtained were analyzed by one way analysis of variance (ANOVA) followed by Tukey post hoc test for multiple comparison using statistical package for social sciences (SPSS) software (Version 20). The differences between means were considered significant at  $p < 0.05$ . All values were expressed as Mean  $\pm$  Standard Error of the Mean (S.E.M.) and presented in tables and figures.

## 3. Results

The extractive value of the methanol tuber extract of *C. alismifolium* was found to be 51.6 g which gave a yield of (5.16 % $^w/w$ ). The preliminary phytochemical test revealed the presence of alkaloids, saponins, flavonoids, glycosides, cardiac glycosides and triterpenes.

### 3.1. HPLC analysis of methanol tuber extract of *Chlorophytum alismifolium*

The HPLC chromatogram of *C. alismifolium* revealed seventeen characteristic peaks of the tuber extract with retention times ranging between 2.093 and 7.409 min. The major peaks presented the following retention times (min): 2.357, 2.712, 2.897 and 4.043 (Fig. 1).

### 3.2. GC-MS analysis of methanol tuber extract of *Chlorophytum alismifolium*

The GC-MS analysis of the tuber extract of *Chlorophytum alismifolium* revealed various bioactive compounds. The active principles with their retention times (RT), concentrations (peak area %) are presented in Table 1.

### 3.3. Acute toxicity studies

The LD<sub>50</sub> of the crude methanol extract of *C. alismifolium* was estimated to be more than 5000 mg/kg in mice and no adverse symptoms or death was recorded in both phases of the experiment.

### 3.4. Antinociceptive studies

The administration of *C. alismifolium* extract at 400 mg/kg significantly ( $p < 0.01$ ) reduced the mean number of acetic acid-induced writhes when compared to distilled water control group. Similarly, the standard agent used (Morphine, 10 mg/kg) significantly ( $p < 0.001$ ) reduced the mean number of writhes when compared to distilled water control group. In addition, the mean number of writhes produced in the morphine group was significantly ( $p < 0.05$ ) lower than that of the extract treated group (Fig. 2).

### 3.5. Mechanistic studies

Administration of CAE significantly ( $p < 0.01$ ) reduced the mean number of writhes in mice when compared to control group. The pretreatment of animals with naloxone + CAE, prazosin + CAE and propranolol + CAE did not produce a significant ( $p > 0.05$ ) increase or decrease in the antinociceptive activity of the extract when compared to CAE-alone treated group. Furthermore, they showed 61.19, 72.01 and 62.01% inhibition of writhes respectively (Table 2). However, the pretreatment of animals with yohimbine + CAE and glibenclamide + CAE significantly ( $p < 0.05$  and  $p < 0.01$  respectively) reduced the antinociceptive activity of the CAE-alone treated group (Table 2).

## 4. Discussion

Phytochemical compounds from medicinal plants such as alkaloids, flavonoids and saponins have proven to be valuable in the management of painful conditions.<sup>15</sup> The preliminary phytochemical screening of *C. alismifolium* revealed the presence of compounds that are corroborative of the findings of Abubakar et al.<sup>7</sup> With tremendous expansion in the use of herbal medicines worldwide, their quality control has been an important concern for both health authorities and the public.<sup>16</sup> Chromatographic fingerprinting has therefore become one of the most useful approaches in quality control of herbal medicines.<sup>17</sup> In this study, the HPLC fingerprint of the tuber extract showed a spectrum profile characteristic of *Chlorophytum alismifolium*. According to Cheng et al.,<sup>18</sup> fingerprinting chromatogram of herbal medicines represent their chemical characteristics and has potential to determine their identity, consistency and authenticity. The GC-MS analysis revealed the presence of bioactive compounds with proven analgesic activities. Some of these compounds include propanoic acid derivatives,<sup>19</sup> pyrrole derivatives<sup>20</sup> and propargylamine derivatives.<sup>21</sup> One of the major compounds present in *C. alismifolium* tuber extract (1,4-Bis(trimethylsilyl)benzene) is a monoterpene; an important class of compounds with analgesic activity.<sup>22</sup> Monoterpenes have been shown to produce their actions through multiple pathways.<sup>22</sup> Citronellal for example is a monoterpene present

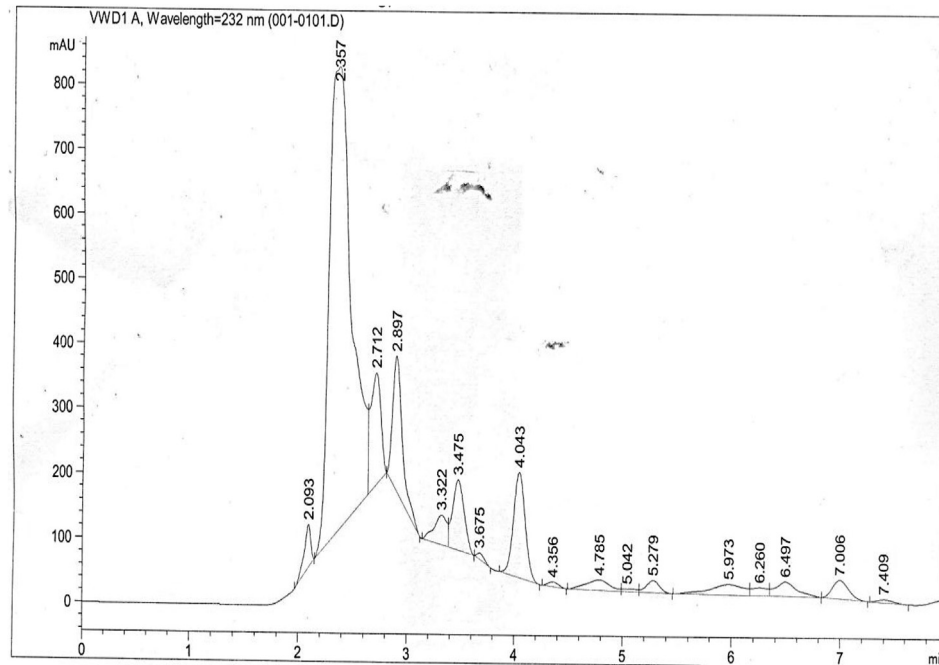


Fig. 1. HPLC chromatogram of methanol extract of *Chlorophytum alismifolium*.

Table 1

Compounds detected in methanol extract of *Chlorophytum alismifolium*.

Compounds	RT	Area %
2-Pentanone, 4-hydroxy-4-methyl-	5.381	2.44
Methylacrylonitrile	9.6307	0.01
2-Propenamide	18.936	0.03
(5R,6R)-3,6-Dimethyl-5-(prop-1-en-2-yl)-6-vinyl-6,7-dihydrobenzofuran-4(5H)-one	38.1693	0.16
Propargylamine	44.4705	0.08
Propane, 1-methoxy-2-methyl-	45.4597	0.05
1H-1,2,3-Triazole	45.7161	0.01
Ethenamine, N-methylene-	49.8925	0.01
Propanoic acid	50.1855	0.03
Pyrrole	51.248	0.03
2-Propynenitrile, 3-fluoro-	51.7242	0.02
13-Tetradecene-11-yn-1-ol	52.3104	0.05
2-Butanamine, (S)-	53.959	0.02
Pyridine, 2,3,4,5-tetrahydro-	55.2412	0.05
Acetonitrile, 2,2'-iminobis-	55.7907	0.02
1,19-Eicosadiene	58.3918	0.03
1,4-Bis(trimethylsilyl)benzene	98.507	96.96

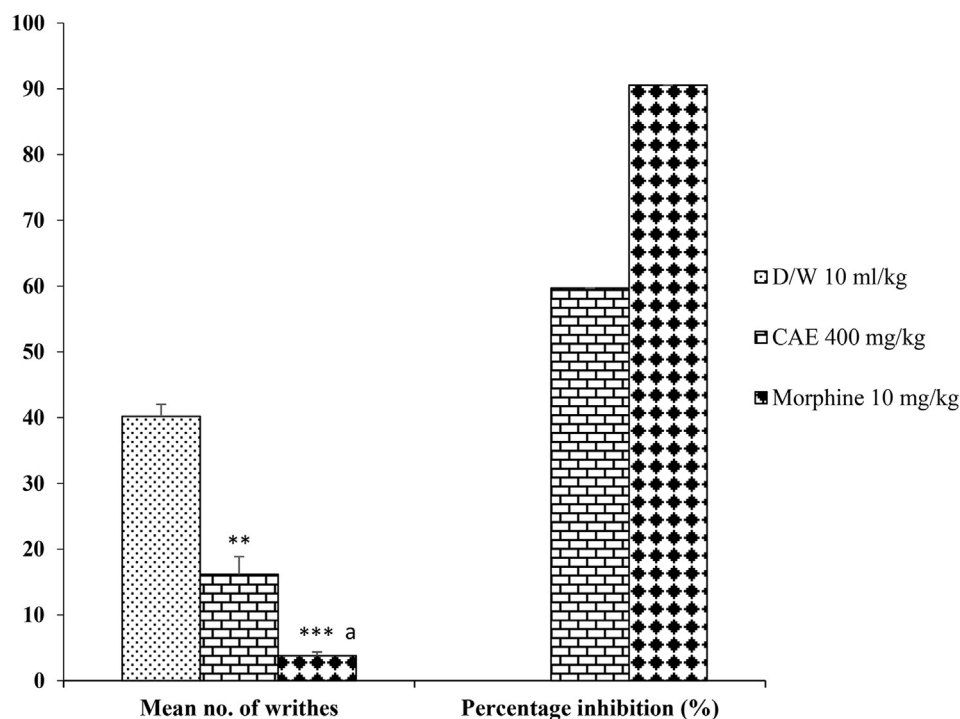
in *Cymbopogon citratus* and *Cymbopogon winterianus*, and its analgesic actions has been shown to be mediated through nitric oxide, cyclic guanosine monophosphate and  $K_{ATP}$  channels (NO-cGMP- $K_{ATP}$  channels).<sup>23</sup> Therefore, it is plausible that the anti-nociceptive actions of *C. alismifolium* could be due to the presence of terpenes amongst other phyto-constituents.

Previous studies on *C. alismifolium* tuber extract established its significant antinociceptive activity in formalin and acetic acid-induced experimental pain models.<sup>7</sup> Consistent with these studies, our results revealed that the administration of *C. alismifolium* extract significantly reduced the number of writhes in mice. Various neurotransmitters and receptor systems modulate pain processes in the central and peripheral nervous systems.<sup>24</sup> These include potassium ion ( $K^+$ ) channels,<sup>25</sup> adrenergic,<sup>26</sup> glutamatergic,<sup>27</sup> and opioidergic systems.<sup>14,23</sup> Pretreatment of mice with yohimbine and glibenclamide significantly increased the antinociceptive activity when compared to *C. alismifolium* alone treated

group. This shows that  $\alpha_2$ -adrenergic receptor and  $K_{ATP}$  channels are possibly involved in the antinociceptive activity of *C. alismifolium*. By contrast, pretreatment with prazosin, naloxone and propranolol did not change the antinociceptive action of *C. alismifolium*, suggesting that  $\alpha_1$ -adrenergic, opioid and  $\beta$  adrenergic receptors may not be implicated in its antinociceptive activity.

The acetic acid-induced writhing test is an established model for screening analgesic agents in mice<sup>28,29</sup> as well as their mechanisms.<sup>14,30</sup> Acetic acid stimulates pain by liberating endogenous substances such as serotonin, histamine, prostaglandins, bradykinins and substance P<sup>31</sup> and stimulates central pain by the activation of mitogen-activated protein (MAP) kinases and microglia in the spinal cord.<sup>30,32</sup> It also modulates central pain through a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems.<sup>31</sup>

The administration of  $\alpha_2$ -adrenoceptor agonists produce antinociception in rodents by inhibiting synaptic transmission in the



**Fig. 2.** Effect of methanol tuber extract of *Chlorophytum alismifolium* on acetic acid-induced writhing test in mice. Values are presented as Mean  $\pm$  S.E.M. <sup>\*\*</sup> =  $p < 0.01$ , <sup>\*\*\*</sup> =  $p < 0.001$  compared to D/W control group, <sup>a</sup> =  $p < 0.05$  compared to CAE group – One way ANOVA followed by Tukey post hoc test;  $n = 6$ ; D/W = Distilled water, CAE = *Chlorophytum alismifolium* extract.

**Table 2**

Effect of different receptor blockers on the antinociceptive activity of methanol tuber extract of *Chlorophytum alismifolium* on acetic acid-induced writhing test in mice.

Treatment	Dose (mg/kg)	Mean number of writhes	Percentage inhibition (%)
D/W	10 ml/kg	40.20 $\pm$ 1.83 <sup>b</sup>	–
CAE 400	400	16.20 $\pm$ 2.65 <sup>**</sup>	59.70
Naloxone + CAE	2	15.60 $\pm$ 5.15 <sup>**</sup>	61.19
Prazosin + CAE	1	11.25 $\pm$ 7.04 <sup>*</sup>	72.01
Yohimbine + CAE	1	36.40 $\pm$ 3.57 <sup>a</sup>	9.45
Propranolol + CAE	20	15.25 $\pm$ 7.47 <sup>**</sup>	62.06
Glibenclamide + CAE	5	39.80 $\pm$ 2.82 <sup>b</sup>	1.00

Values are presented as Mean  $\pm$  S.E.M; <sup>\*</sup> =  $p < 0.05$ ; <sup>\*\*</sup> =  $p < 0.01$  compared to distilled water control group; <sup>a</sup> =  $p < 0.05$ ;  $p < 0.01$  compared to CAE group – One way ANOVA followed by Tukey post hoc test;  $n = 6$ ; D/W = Distilled water, CAE = *Chlorophytum alismifolium* extract.

dorsal horn of the spinal cord and there is an evidence that stimulation of the descending noradrenergic system results in the activation of spinal  $\alpha_2$ -adrenoceptor and antinociception.<sup>33,34</sup> All the three  $\alpha_2$ -adrenoceptor subtypes have also been reported to act as presynaptic inhibitory feedback receptors to control the release of catecholamines from adrenergic neurons and are linked to antinociceptive process.<sup>35,36,37</sup> In this study, pre-treatment of mice with yohimbine (a presynaptic  $\alpha_2$ -adrenergic receptor antagonist) significantly attenuated the antinociceptive activity of *C. alismifolium* extract. This implies that  $\alpha_2$ -adrenergic receptor plays a role in its antinociceptive activity.

$K_{ATP}$  channels are widely distributed in the central and peripheral nervous systems<sup>38</sup> and are involved in different neuronal activities such as neuroprotection, control of neurotransmitter release and regulation of membrane excitability.<sup>39</sup> The loss of  $K_{ATP}$  current contributes to neuropathic pain<sup>39</sup> while the opening of  $K_{ATP}$  channels and the consequent cellular hyperpolarization are involved in the antinociceptive effects of drugs with different mechanisms of action.<sup>14,40</sup> In our study, glibenclamide (an inhibitor of  $K_{ATP}$  channels) significantly attenuated the antinociceptive

activity of *C. alismifolium* extract, thus validating the important role of  $K_{ATP}$  channels in its antinociceptive activity.

The analgesic activity of *C. alismifolium* extract may not be mediated through the opioidergic,  $\alpha_1$ -adrenergic and  $\beta$ -adrenergic pathways. This is because pretreatment of mice with naloxone (a non-selective opioid receptor antagonist), prazosin (an  $\alpha_1$ -adrenoceptor antagonist) and propranolol (a non-selective  $\beta$ -adrenoceptor antagonist) each did not significantly alter the antinociceptive action of *C. alismifolium* extract when the mice were pretreated with either of the antagonists.

## 5. Conclusion

The data presented in this study revealed that  $\alpha_2$ -adrenergic receptor and  $K_{ATP}$  channels play important roles in the antinociceptive activity of methanol tuber extract of *C. alismifolium*.

## Conflicts of interest

The authors declared no conflict of interest.



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