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Elucidation of the possible mechanism of analgesic action of methanol stem bark extract of *Uapaca togoensis* pax in mice

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ABSTRACT

Ethnopharmacological relevance: *Uapaca togoensis* is a medicinal plant used traditionally in Africa for the treatment of rheumatism, epilepsy, cough, pneumonia, vomitting and fever. Previously, the analgesic activity of its methanol stem bark extract has been scientifically demonstrated. However, the mechanism responsible for this activity remains to be investigated.

Aim of the study: To elucidate the possible mechanism(s) through which the methanol stem bark extract of *Uapaca togoensis* (MEUT) exhibits analgesic activity in mice.

Materials and methods: Analgesic activity of MEUT was evaluated using acetic acid-induced abdominal writhing test in mice at doses of 250, 500 and 1000 mg/kg orally. For the mechanistic studies, mice were pre-treated with Naloxone (2 mg/kg), Atropine (1 mg/kg), Yohimbine (1 mg/kg), Glibenclamide (10 mg/kg), Prazosin (1 mg/kg) and Yohimbine (1 mg/kg) 15 min prior to MEUT (1000 mg/kg) administration, then assessed using AAWT 1 h later. Data was analysed using One way Anova followed by Bonferroni post hoc test.

Results: The extract (at the doses of 250, 500 and 1000 mg/kg) and morphine (10 mg/kg) significantly ($p < 0.05$) decreased the number of abdominal writhes. Naloxone (opioid receptor antagonist), Atropine (muscarinic receptor antagonist) and Glibenclamide (ATP-sensitive K+ channel blocker) significantly ($p < 0.05$) reversed the analgesic effect of MEUT. On the other hand, Prazosin and Yohimbine (α_1 and α_2 receptor antagonists respectively) had no effect on the analgesic action of MEUT.

Conclusion: The results obtained from this study suggests the possible involvement of opioidergic, cholinergic and sensitive potassium ATP channel pathways in the analgesic activity of the methanol stem bark extract of *Uapaca togoensis*.

1. Introduction

Pain is a pathological condition which negatively impacts the quality of life of individuals with an enormous economic implication (Uritu et al., 2018). Defined by the International Society for the study of pain as an unpleasant sensory emotional experience associated with actual or potential tissue damage, pain remains one of the oldest challenges in medical history (Raffaeli and Arnaudo, 2017; IASP, 2018). Pain is a common symptom of many diseases and may also result from surgical interventions or trauma (Mohammadi, 2018). The concept of pain is multidimensional involving several components namely sensory, physiological, cognitive, affective, behavioral and spiritual components (WHO, 2012). Presently pain is not only a major problem globally, it is also responsible for the increasing number of disabilities worldwide (Gedin et al., 2017).

Numerous synthetic analgesic drugs have been developed and utilized in pain management, however drugs like the opioids (morphine) and non-steroidal anti-inflammatory drugs have been associated with side effects including addiction, withdrawal, respiratory depression, gastrointestinal erosion, peptic ulcers, nephrotoxicity, leukopenia and allergic manifestations (Yougbare-Ziebrou et al., 2016; Tatiya et al., 2017). For these reasons, there has been a significant increase in the search for new drugs with better efficacy and safety with focus on medicinal plants.

Medicinal plants since time immemorial have served as therapeutic alternatives for the treatment of many ailments including fever, pain, arthritis, malaria and migraine (Lalrinzuali et al., 2016; Rauf et al., 2017). Current researches promote the use of these medicinal plants for the treatment and prevention of diseases because the plants are available, cheap and are thought to be safer than synthetic drugs (Nasir,

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2016). These researches have gone further to investigate how some of these medicinal plants block or antagonize pathways that are involved in the mechanism of pain. This has resulted in the screening of compounds such as nerve growth factor (NGF) antagonists (Watson et al., 2008), selective Na channel blockers (Jarvis et al., 2007) and bradykinin receptor antagonists (Rodger, 2009) for possible development as novel analgesic drugs to replace or augment the already available analgesic agents (Boakye-Gyasi et al., 2017). *Uapaca togoensis* a plant belonging to the family Euphorbiaceae has been traditionally used to treat several ailments including epilepsy, vomiting, rheumatism, fever, fatigue, cough and pneumonia (Koné et al., 2007). An evergreen tree that usually grows up to about 20 m tall, it has large leaves with fruits ellipsoidal in shape (Adjanohoun et al., 1989; Bretler, 2013). The analgesic activity of the methanol stem bark extract has been scientifically validated previously (Olorukooba et al., 2015). It has also been reported to possess antibacterial (Koné et al., 2004), larvicidal (Azokou, 2013), antifungal and antimicrobial (Omachi et al., 2015; Seukep et al., 2016) and anticancer (Kuete et al., 2015) activities. Despite the growing number of pharmacological investigations conducted in these last years, there is a lack of more substantial data on the possible mechanisms of analgesic action of the plant. The present study therefore aimed at evaluating the possible mechanism(s) responsible for the analgesic activity of methanol stem bark extract of *Uapaca togoensis* in mice.

2. Materials and Methods

2.1. Plant collection and extraction

The plant materials of *Uapaca togoensis* including leaves, fruits and stem were collected in June 2015 and taken to Herbarium Section of Department of Botany, Ahmadu Bello University Zaria where it was identified and authenticated by a botanist. A voucher specimen number 1279 was deposited for future reference.

The stem bark of the plant was taken to and dried in the Department of Pharmacognosy and Drug Development. It was then pulverised using mortar and pestle. About 1000 g of pulverised materials were extracted using maceration with 5 L of methanol (70% v/v). The solution was concentrated on water bath (45^0), after which the extract was stored in desiccator. Aqueous solutions of the extract were freshly prepared for each study using distilled water.

2.2. Animals

Swiss Albino mice (both sexes) weighing between 18 and 22 g were obtained from the Animal House Facility of Pharmacology and Therapeutics Department, Ahmadu Bello University Zaria. The animals were housed in clean cages and maintained under natural day amd light cycle and fed with standard rodent pellet diet and water *ad libitum*. Animals were used in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals by the National Institute of Health Principles, 1998.

2.3. Chemicals and drugs

The followings are some of the chemicals and drugs used for the experiment.

Methanol (Sigma-Aldrich), Naloxone hydrochloride, Metergoline, Prazosin, Atropine, Glibenclamide, Yohimbine hydrochloride (Abcam Plc, Cambridge, UK), Acetylsalicylic acid (Bayer, Leverkusen, Germany), Distilled water.

2.4. Acute toxicity study in mice

Oral median lethal dose (LD_{50}) was determined using Lorke's method Lorke, 1983. A total of 13 mice were used in the biphasic study. In the first phase, 9 mice divided into 3 groups of 3 mice each were treated with the extract at doses of 10, 100 and 1000 mg/kg orally. The mice were then observed for 24 h for any signs of toxicity or mortality before proceeding to the next phase. In the second phase, 4 groups containing one mouse each were orally administered with the extract at doses of 1200, 1600, 2900 and 5000 mg/kg. Mice were also observed for 24 h for any signs of toxicity or mortality. The oral median lethal dose (LD_{50}) was determined by calculating the lowest dose that caused death and the highest dose for which the animals survived.

2.5. Acetic acid-induced writhes in mice

Thirty mice were randomized into 5 groups consisting of 6 mice each. The first and last groups were treated with distilled water (10 ml/kg) and acetyl salicylic acid (ASA, 300 mg/kg) orally. While the second, third and fourth groups were treated with the extract at doses of 250, 500 and 1000 mg/kg respectively also through the oral route. One-hour post treatment 0.6% v/v of acetic acid (10 ml/kg) were injected intraperitoneally into each mouse to induce abdominal writhes. Five minutes after the injection of acetic acid, the number of writhes was counted for each mouse for a period of 10 min. The ability of the extract to cause a reduction in abdominal writhes was expressed as percentage inhibition of writhing and calculated using the following formula below:

$$\text{Percentage inhibition} = \frac{\text{Average number of writhes (control)} - \text{Average number of writhes (test)}}{\text{Average number of writhes (control)}} \times 100$$

(Koster et al., 1959).

2.6. Mechanistic studies

The participation of the opioidergic, adrenergic, cholinergic and K channel blocker pathways in the analgesic activities of the methanol stem bark extract of *Uapaca togoensis* (MEUT) was investigated using mouse model of acetic acid induced writhing as previously described by Rangel et al. (2012).

2.6.1. To determine the possible involvement of opioidergic pathway

The possible involvement of opioidergic pathway in the analgesic action produced by MEUT was investigated using the acetic acid-induced abdominal writhes test in mice. Thirty (30) mice were grouped into 6 groups each consisting of 5 mice. Groups 1, 2 and 3 were treated with distilled water (10 ml/kg), MEUT (1000 mg/kg) and morphine (10 mg/kg) orally respectively. Group 4 and 5 were pre-treated with naloxone (2 mg/kg, *ip*) a non-selective opioid receptor antagonist. Fifteen minutes later, group 4 and 5 were treated with MEUT (1000 mg/kg) and morphine (10 mg/kg) orally respectively. At the end of 1 h, mice in all groups were assessed using acetic acid-induced writhing test.

2.6.2. To investigate the possible involvement of cholinergic system

To investigate the possible involvement of the cholinergic system in the analgesic action of MEUT, 30 mice divided into 5 groups of 6 mice each were used. The first, second and third groups received distilled water (10 ml/kg), MEUT (1000 mg/kg) and morphine (10 mg/kg)

orally respectively. The fourth and fifth groups were pre-treated with atropine (1 mg/kg, *ip*) a muscarinic receptor antagonist 15 min prior to administration of MEUT (1000 mg/kg) and morphine (10 mg/kg) orally respectively. Mice in all the groups were assessed 1 h later using acetic acid-induced writhing test.

2.6.3. To investigate the possible involvement of K_{ATP} channel blocker pathway

To investigate the possible involvement of the K_{ATP} Channel blocker system in the analgesic action of MEUT, 30 mice divided into 5 groups of 6 mice each were used. The first, second and third groups received distilled water (10 ml/kg), MEUT (1000 mg/kg) and morphine (10 mg/kg) orally respectively. The fourth and fifth groups were pre-treated with glibenclamide (5 mg/kg, *ip*) a K_{ATP} Channel blocker 15 min prior to administration of MEUT (1000 mg/kg) and morphine (10 mg/kg) orally respectively. Mice in all the groups were assessed 1 h later using acetic acid-induced writhing test.

2.6.4. To investigate the possible involvement of noradrenergic system

To investigate the possible involvement of the noradrenergic system in the analgesic action of MEUT, 42 mice grouped into 7 groups of 6 mice each were used. The first, second and third group received distilled water (10 ml/kg), MEUT (1000 mg/kg) and morphine (10 mg/kg) orally respectively. The fourth and fifth groups were pre-treated with prazosin (1 mg/kg, *ip*) an α1 adrenergic antagonist 15 min prior to administration of MEUT (1000 mg/kg) and morphine (15 mg/kg) orally respectively. The sixth and seventh groups were pre-treated with yohimbine (1 mg/kg, *ip*) an α2 adrenergic antagonist, 15 min later, MEUT (1000 mg/kg) and morphine (10 mg/kg) were administered orally respectively. Mice in all the groups were assessed 1 h later using acetic acid-induced writhing test.

2.7. Statistical analysis

Values were expressed as mean ± SEM and differences analysed by One-Way Analysis of Variance (ANOVA) followed by Dummett post hoc test using SPSS version 20.0. A value of $p \leq 0.05$ was considered as statistically significant.

3. Results

3.1. Acute toxicity (LD_{50}) of methanol stem bark extract of Uapaca togoensis

The LD_{50} of methanol stem bark extract of *Uapaca togoensis* was estimated to be greater than 5000 mg/kg orally in mice.

3.2. Analgesic studies

Effect of Methanol Stem Bark Extract of *Uapaca togoensis* on Acetic acid-induced Writhes in Mice.

The methanol extract of *U. togoensis* significantly ($p < 0.05$) and dose dependently decreased the number of abdominal writhes as compared to the distilled water treated group. A significant ($p < 0.05$) reduction in abdominal writhes was also observed in the morphine treated group (Fig. 1).

3.3. Mechanistic studies

3.3.1. Involvement of the opioidergic pathway

The extract (1000 mg/kg) and morphine (10 mg/kg) significantly ($p < 0.05$) reduced the mean number of abdominal writhes as compared to distilled water treated group. However, naloxone significantly ($p < 0.05$) reversed the effect of both extract and morphine by increasing the mean number of abdominal writhes (Fig. 2).

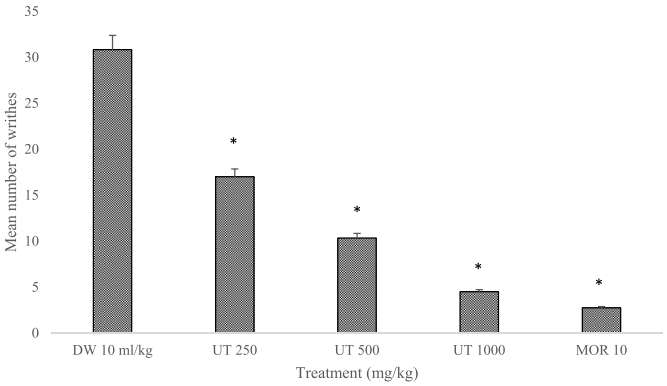

Fig. 1. Effect of Methanol Stem Bark Extract of *Uapaca togoensis* on Acetic acid-Induced Abdominal Writhes in Mice
Each column represents the Mean ± SEM, n = 6. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, * = $p \leq 0.05$ significantly different from DW group; UT= *Uapaca togoensis* (1000 mg/kg, *po*); MOR = Morphine (10 mg/kg, *po*), DW = Distilled water (10 ml/kg, *po*).

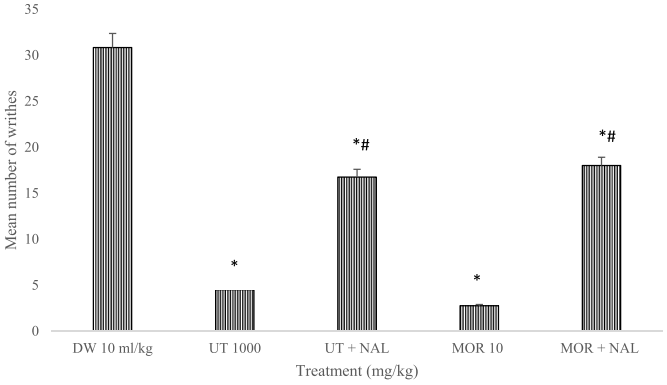

Fig. 2. Effect of Naloxone on Analgesic Activity of Methanol Stem Bark Extract of *Uapaca togoensis* in Acetic acid-Induced Writhes Test in Mice
Each column represents the Mean ± SEM, n = 6. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, * = $p \leq 0.05$ significantly different from DW group; # = $p \leq 0.05$ significantly different from DW group UT= *Uapaca togoensis* (1000 mg/kg, *po*); MOR = Morphine (10 mg/kg, *po*), DW = Distilled water (10 ml/kg, *po*), NAL= Naloxone (2 mg/kg, *ip*).

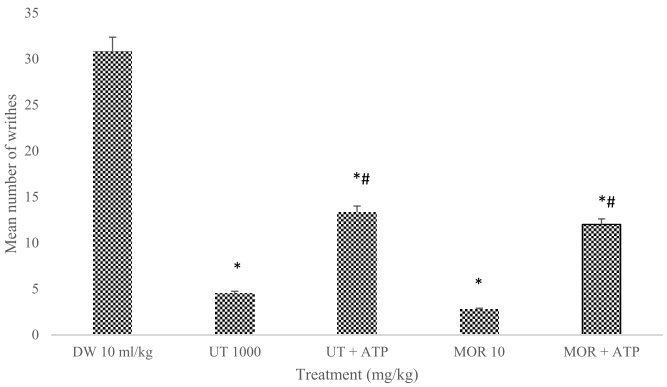

Fig. 3. Effect of Atropine on Analgesic Activity of Methanol Stem Bark Extract of *Uapaca togoensis* in Acetic acid-induced Writhes Test in Mice
Data are Mean ± SEM, n = 6, analysed using One-way ANOVA followed by Bonferroni post hoc test, * = $p \leq 0.05$ significantly different from DW group; # = $p \leq 0.05$ significantly different from UT treated group; UT = *Uapaca togoensis* (1000 mg/kg, *po*); MOR = Morphine (10 mg/kg, *po*), DW = Distilled water (10 ml/kg, *po*), ATP = Atropine (1 mg/kg, *ip*).

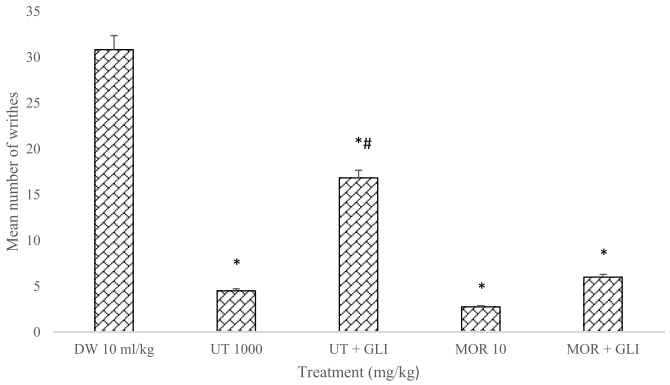

Fig. 4. Effect of Glibenclamide on Analgesic Activity of Methanol Stem Bark Extract of *Uapaca togoensis* in Acetic acid-induced Writhes Test in Mice

Data are Mean ± SEM, n = 6, analysed using One-way ANOVA followed by Bonferroni post hoc test, * = $p \leq 0.05$ significantly different from DW group; $^{\#}p \leq 0.05$ significantly different from UT treated group; UT = *Uapaca togoensis* (1000 mg/kg, *po*); MOR = Morphine (10 mg/kg, *po*), DW = Distilled water (10 ml/kg, *po*), GLI = Glibenclamide (10 mg/kg, *ip*).

3.3.2. Involvement of the cholinergic pathway

Pre-treatment with atropine significantly ($p \leq 0.05$) decreased the mean number of abdominal writhes of mice treated with methanol stem bark extract of *Uapaca togoensis* (1000 mg/kg) and morphine (10 mg/kg) (Fig. 3).

3.3.3. Involvement of K_{ATP} channel blocker pathway

MEUT (1000 mg/kg) and morphine (10 mg/kg) significantly ($p < 0.05$) decreased the mean number of abdominal writhes as compared to the distilled water treated group. The reduction in the number of abdominal writhes produced by the extract was reversed by glibenclamide (10 mg/kg). However, pre-treatment with glibenclamide did not significantly change the mean number of abdominal writhes of mice treated with morphine (Fig. 4).

3.3.4. Involvement of noradrenergic system

There was significant ($p < 0.05$) reduction in mean number of abdominal writhes in mice treated with either methanol stem bark extract of *U. togoensis*, morphine, extract pre-treated with prazosin and morphine pre-treated with prazosin (α1 adrenergic antagonist) as compared to the distilled water treated group (Fig. 5).

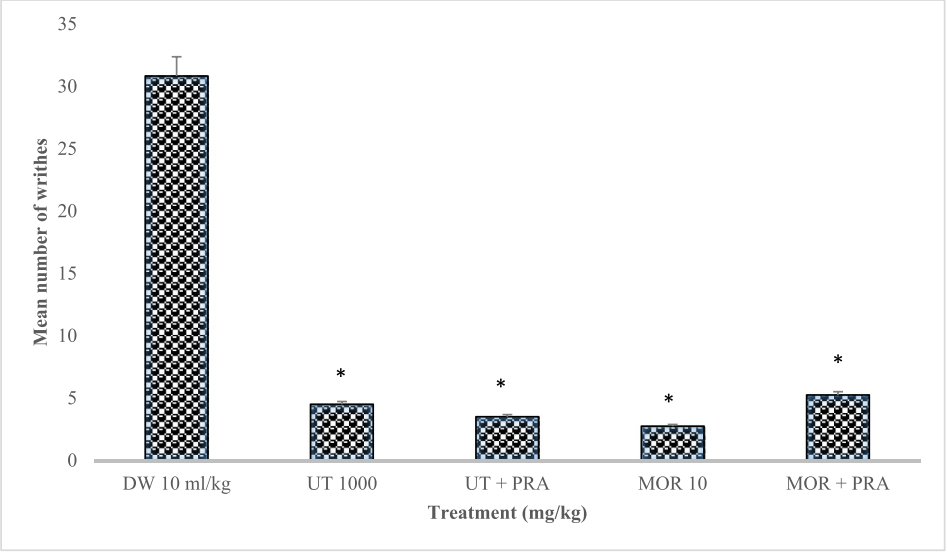

Fig. 5. Effect of Prazosin on Analgesic Activity of Methanol Stem Bark Extract of *Uapaca togoensis* in Acetic acid-Induced Writhes Test in Mice.

Each column represents the Mean ± SEM, n = 6. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, * = $p \leq 0.05$ significantly different from DW group; UT = *Uapaca togoensis* (1000 mg/kg, *po*); MOR = Morphine (10 mg/kg, *po*), DW = Distilled water (10 ml/kg, *po*), PRA = Prazosin (1 mg/kg, *ip*).

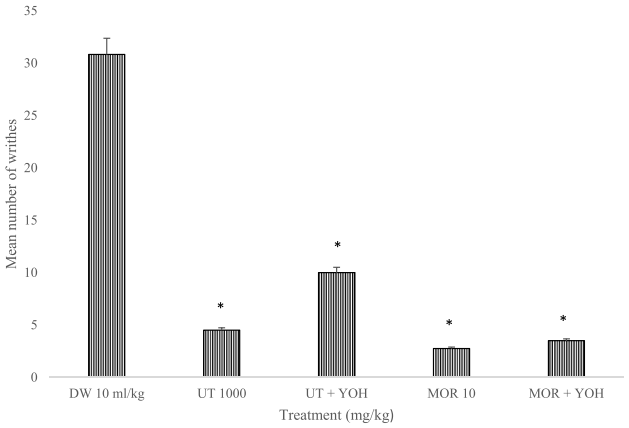

Fig. 6. Effect of Yohimbine on Analgesic Activity of Methanol Stem Bark Extract of *Uapaca togoensis* in Acetic acid-Induced Writhes Test in Mice

Each column represents the Mean ± SEM, n = 6. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, * = $p \leq 0.05$ significantly different from DW group; UT = *Uapaca togoensis* (1000 mg/kg, *po*); MOR = Morphine (10 mg/kg, *po*), DW = Distilled water (10 ml/kg, *po*), YOH = Yohimbine (1 mg/kg, *ip*).

Similarly, there was a significant decrease between distilled water treated group and the groups treated with extract, extract pre-treated with yohimbine (α2 adrenergic antagonist), morphine and morphine pre-treated with yohimbine. However, there was an increase in mean number of abdominal writhes in the extract pre-treated with yohimbine as compared to extract only treated group but this was not statistically significant (Fig. 6).

4. Discussion

The methanol stem bark extract of *Uapaca togoensis* has been reported by a previous study to exert significant analgesic effect in both chemical- and thermal-induced pain test models; indicating possible involvement of peripheral and central mechanisms in its analgesic action (Olorukooba et al., 2015). In the present study, the possible mechanisms of analgesia of MEUT were further investigated. The findings from the study showed the involvement of the opioidergic, cholinergic and potassium ATP channel pathways in the analgesic effect produced by the extract with no involvement of the adrenergic pathways.

Numerous evidence has shown the participation of many other

pathways in the multiple actions of analgesics during pain. These pathways include monoaminergic pathways (noradrenergic, cholinergic, and serotonergic) and/or the purinergic pathway (adenosinergic system) (Zakaria et al., 2018). To further identify other pathways possibly involved in the pain-relieving effect of *Uapaca togoensis* stem bark extract, mice were treated with either with the extract or morphine in the presence of the following antagonists; naloxone, atropine, glibenclamide, prazosin and yohimbine. The ability of the antagonists to reverse the pain-relieving activity of the extract or morphine was then assessed using acetic acid-induced abdominal writhing test in mice.

Administration of the methanol stem bark extract of *Uapaca togoensis* reduced the number of abdominal writhing's in mice. The acetic acid-induced abdominal writhing test is a sensitive model used for screening compounds for peripheral analgesic activity. (Collier et al., 1968). The injection of acetic acid into mice leads to the release of several pro-inflammatory mediators (i.e., bradykinin, serotonin, histamine, substance P or prostaglandins (PGE2 and PGF2α) which activate the peripheral nociceptive neurons within the peritoneal cavity to cause painful sensations (Ribeiro et al., 2000). MEUT caused a significant dose-dependent decrease in the number of acetic acid-induced abdominal writhes in comparison to control. This observation suggests that the extract possesses peripherally-mediated analgesic activity induced through different mechanisms and probably linked to the inhibition of prostaglandin synthesis, GMP-ATP-sensitive K+ channel cascade, serotonergic pathway, cholinergic pathway, adrenergic pathway and inhibition of cytokine production (Hamza and Dionne, 2009). However, the extract may also be acting centrally through reducing the sensory effect of acetic acid (Stevenson et al., 2006).

Generally, opioid analgesics act by binding to opiate receptors in the midbrain periaqueductal grey area leading to the suppression of responses of the spinal neurons to noxious stimuli by the activated descending brain stem inhibitory systems (Abbott et al., 1982). To determine the involvement of the opioidergic pathway in the analgesic activity of MEUT, naloxone (a non-selective opioid antagonist) which acts by blocking spinal and supraspinal opioid receptors in the central nervous system was used. In the study, pre-treatment with naloxone, reversed the analgesic effects of the extract. This suggests the involvement of opioid receptors and/or endogenous opioids in the analgesic effects of the extract.

To investigate the involvement of the cholinergic system in the analgesic effect of MEUT, animals were pre-treated with atropine. Atropine is a non-selective anticholinergic or an antimuscarinic agent that antagonizes the muscarinic actions of acetylcholine and other choline esters (Howland, 2009). In this study, pre-treatment with atropine resulted in the reversal of analgesic activity of MEUT. This indicated the involvement of cholinergic pathways in the analgesic activity of the extract.

The generation of many pain signals in the human nervous system is mediated by ion channels which are essential for controlling neuronal excitability (Zakaria et al., 2018). The opening of K^+ channels produces analgesia by reducing neuronal excitability and inhibiting the release of various neurotransmitters in the spinal cord (Palkovits, 2000). Pre-treatment of mice with glibenclamide (a specific ATP-sensitive K+ channel blocker) was shown to inhibit the analgesic activity of MEUT. The ability of glibenclamide to inhibit the analgesic effect of morphine previously reported in other studies was also observed in this study (Longhi-Balbinot et al., 2011; Zakria et al., 2018). The reversal in the analgesic effect of MEUT after glibenclamide pre-treatment thus suggests possible involvement of ATP-sensitive K^+ channels in the analgesic effect of MEUT. Since the opening of ATP-sensitive K+ channel has been reported to participate in opioid-mediated analgesia (Rodrigues and Duarte, 2000), this correlates well with previous study demonstrating the involvement of MEUT in opioid receptor system.

The noradrenergic system has been implicated in nociception at spinal and supraspinal levels mediated through activation of α-adrenoceptors and descending inhibitory pathways (Millan, 2002). This system has also been shown to be involved in the mechanism of chronic pain (Roczniak et al., 2013). In this study, pretreatment of mice with prazosin (a selective α1-adrenoceptor antagonist) and yohimbine (a selective α2-adrenoceptor antagonist) failed to reverse the analgesic activity of the extract. These suggest the non-involvement of the α1-adrenoceptor and α2-adrenoceptor pathways in the analgesic activity of the extract.

5. Conclusion

The present study has demonstrated the possible mechanisms of analgesia of MEUT to involve activation of the opioidergic and cholinergic receptors, in addition to opening of ATP-sensitive K^+-channels.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2019.112156.

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Conflicts of interest

The authors declare no conflict of interest.

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