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*Research Article*

# Evaluation of Analgesic Potential of *Shumanniophyton magnificum* Extract on Albino Rats

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## ABSTRACT

*Shumaniophyton magnificum* is usually used in African folk medication to treat various diseases, especially fever, malaria, and pain. Pain is a well-known indicator of illness, and the medications used to treat pain are called analgesics. The primary issue with these medications continues to be their side effects. Natural herbs are safer substitutes. This study explored the analgesic potentials of the methanol extract of *S. magnificum* leaf using three distinct *in vivo* analgesic models: the Acetic Acid-Induced Writhing, Hot Plate, and Tail Flick Response Assessments. When compared to aspirin-treated groups, the *S. magnificum* extract significantly ($p<0.05$) and dose-dependently reduced the number of acetic acid-induced writhes in rats. In the hot plate test, the extract significantly ($p < 0.05$) increased the latency time to thermal pain in comparison to groups that received pentazocine treatment. When compared to groups treated with pentazocine, the extract significantly ($p<0.05$) increased the pain reaction time of treated mice to thermal stimuli in a dose-dependent manner. In summary, the study's outcomes supporting the traditional use of *S. magnificum* for pain relief came from the extract's demonstrated pain-relieving properties.

**Keywords**: *Shumaniophyton magnificum, Analgesic, Pain, NSAID*

## INTRODUCTION

According to Bahmani *et al.* (2014), pain is an unpleasant mental and responsive feeling that may cause real or likely tissue damage. Pain can be brought on by a variety of things, such as electrical current, traction, toxic heat, necrosis, inflammation, cuts, and spasms. Debilitating discomfort, pain is associated with a variety of illnesses. One of the most important therapeutic objectives for pain management is pain control (Harchaoui *et al.*, 2018). While most people believe that pain is a defense mechanism that happens when tissue is damaged and the person reacts by blocking out pain stimuli, this is not always the case (Baradaran *et al.*, 2013), in severe cases it reduces quality of life and hinders social functioning.

Millions of people with a variety of painful, inflammatory diseases hope to find treatments that work and have fewer adverse effects (Bahmani *et al.*, 2014). Non-steroid anti-inflammatory drugs are helpful, but after extended period of time, they are linked with major side effects, with stomach ulcers, intestinal damage, cardiovascular risks, hepatotoxicity, and renal failure (Pelletier *et al.*, 2016). Opioids are strong analgesics with a central action that raises the spinal level pain threshold. Examples of opioids include morphine, codeine, and pethidine. According to Manglik *et al.* (2016), they are connected to undesired behavioral traits like physical dependence, the development of tolerance, and respiratory depression, after which there is a shift to herbal

medicine. It has been suggested that medicinal plants have strong, naturally occurring compounds that can be used to treat or prevent conditions related to pain. Herbal medicines are used all over the world, and scientists are constantly searching for new plants with better biological properties.

In African traditional medicine, *S. magnificum* (Rubiaceae), usually identified as "mgba mmiri" in the Igbo dialectal of Nigeria, is used to treat a range of ailments (Joshua *et al.*, 2020). A peptide has been isolated from the aqueous extract of the bark of S. magnificum, the isolated peptide displayed dose-related inhibitions of the properties of cardiotoxin and total venom of cobra species (Houghton *et al.*, 1992) suggesting snakebites treatment, however there is no scientific confirmation backing up the use of its leaves as a pain reliever. The objective of this paper is to study the efficacy of *S. magnificum* leaf on thermal-induced pain model in experimental animals.

## MATERIALS AND METHODS

### Plant material collection and preparation

Fresh leaves of *S. magnificum* were procured from Ukehe town located in the Igbo-Etiti Local Government of Enugu State, Nigeria. The identification of the leaves was done at the International Center for Ethnomedicine and Drug Development (INTERCEDD) in Nsukka, Enugu State, Nigeria; for reference purposes, voucher number INTERSED/837 was entered. The leaves were crushed into a fine powder using a milling machine after being permitted to dry at room temperature. A specified quantity of ground leaves (1974.92 g) was cold soaked for 48 hours at room temperature (26–28 °C) in order get the methanol extract. Once Whatman No. 1 filter paper was used for filtering, the macerate was concentrated using a vacuum rotary evaporator set at 40–60°C. The resulting *S. magnificum* methanol extract was kept in an airtight plastic bottle in a fridge (4 °C).

### Experimental animals

The University of Nigeria Nsukka's Animal House, housed in the department of zoology, provided all of the adult albino rats that were used. Animals were acclimatized for 14 days under standard laboratory environment and maintained *ad libitum* on food and water before use. The Institutional Evaluation Board (Ethics and Biosafety Committee, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria) granted ethical consent for the use of animals; the approval number for this board is UNN/FBS/EC/1008.

### Acetic acid-induced writhing test

The procedures outlined by Taber *et al.* (1969) and Koster *et al.* (1959) were applied. Twenty rats stayed fasted for 6 h and distributed into five groups of four animals per group. Groups 3-5 received the extract intraperitoneally (100, 200 and 400 mg/kg b.w., respectively) each receiving 0.5ml of extract solution while group 1 (vehicle control) received 1 ml/kg b.w. of 3% Tween 80 and group 2 (treated control) received 100 mg/kg b.w of aspirin. Tween 80 was used to prepare the solution for all the groups including the control. Thirty (30) minutes after oral administration of the extract and the drug, 1% acetic acid solution (0.1 ml/kg) was given intraperitoneally to induce pain which was manifested in abdominal constrictions (writhes). After 5 min, each group of rats was monitored visually for the number of writhes for a period of 30 minutes. According to Shreedhara *et al.* (2009), the percentage protection against writhing served as a measure of analgesic activity and was computed using the following formula:

$$\%\ Analgesic\ Activity = \frac{Mean\ writing\ count\ (control\ group - treated\ group)}{Mean\ writing\ count\ of\ control\ group} \times 100$$

### Hot plate test

Twenty rats were fasted for 6 h and divided into five groups of four rats per group. Groups 3-5 received intraperitoneally the extract (100, 200 and 400 mg/kg b.w., respectively) while groups 1 (vehicle control) and 2 (treated control) received 2 ml/kg b.w. 3% Tween 80 and pentazocine (10 mg/kg b.w.) respectively. After 30 minutes of treatment, each rat was gently placed on a hot plate kept at 55 ± 0.5 0C and the period mandated by the rat to lick or jump the paw was taken as a response (Turner, 1965). According to Neto *et al.* (2005), the latency response or cutoff time remained 15 seconds. To prevent tissue damage, there is no record of any animal that stayed past the cutoff time. The method was used to determine the percentage rise in response time or inhibition of pain threshold (Dash et al., 2015).

$$\%\ Elongation = \frac{Latency\ response\ (test) - Latency\ response\ (control)}{Latency\ response\ (test)} \times 100$$

### Tail flick response test

Adzu *et al.* (2003) describe how the experiment was conducted: the time of tail withdrawal from hot water was measured. After a 12-hour fast, about 20 rats were split up into five groups, each with four animals. Groups 1 (vehicle control) and 2 (treated control) received 2 ml/kg b.w. of 3% Tween 80 and pentazocine (10 mg/kg b.w.) respectively, while groups 3-5 received the extract intraperitoneally at doses of 100, 200, and 400 mg/kg b.w. After receiving different dosages of extract, 3% Tween 80, and pentazocine (10 mg/kg b.w.) for an hour, the tails of each rat were immersed in a bath of hot water kept at 50±1°C for about 2 cm. Every rat was measured for pain reaction time (PRT), or the duration of time it took for the rat to flick its tail.

$$\text{Percentage increase in PRT} = \frac{\text{PRT in test/standard} - \text{PRT in control}}{\text{PRT in control}} \times 100$$

## RESULTS AND DISCUSSION

### Results

**Table 1**. Effect of Methanol Leaf Extract of *S. magnificum* on Acetic Acid-Induced Writhing in Rats

| Groups | Treatment | No of writhes per 30min | % Inhibition |
|---|---|---|---|
| **1** | 2 ml/kg of 3% Tween 80 | 91.25±5.38$^{d}$ | |
| **2** | 100 mg/kg Aspirin | 41.75±6.85$^{a}$ | 54.25 |
| **3** | 100 mg/kg Extract | 65.25±7.04$^{c}$ | 28.49 |
| **4** | 200 mg/kg Extract | 54.50±3.87$^{b}$ | 40.27 |
| **5** | 400 mg/kg Extract | 45.00±2.94$^{a}$ | 50.68 |

The values are shown as mean ± SD, with n = 4.
Mean values containing different alphabetic letters in the column exhibit a significant difference ($p < 0.05$).

**Table 2**. Effect of Methanol Leaf Extract of *S. magnificum* on Analgesic Pain Using Hot Plate Test in Rats

| Groups | Dose | Pre-treatment (sec) | Post-treatment (sec) | % inhibition |
|---|---|---|---|---|
| **1** | 2ml/kg of 3% Tween 80 | 2.88±0.50 | 2.88±0.50$^{a}$ | |
| **2** | 10 mg/kg Pentazocine | 3.07±0.02 | 9.48±0.81$^{c}$ | 69.62 |
| **3** | 100mg/kg Extract | 2.63±0.57 | 5.72±1.21$^{b}$ | 49.65 |
| **4** | 200mg/kg Extract | 2.85±0.50 | 6.89±0.88$^{b}$ | 58.20 |
| **5** | 400mg/kg Extract | 3.03±0.02 | 8.65±1.23$^{c}$ | 66.71 |

The values are shown as mean ± SD, with n = 4.
Mean values containing different alphabetic letters in the column exhibit a significant difference ($p < 0.05$).

**Table 3**. Effect of Methanol Leaf Extract of *S. magnificum* on Tail Flick Response in Rats

| Groups | Dose | Pain Reaction Time (PRT) (sec) | % inhibition |
|---|---|---|---|
| **1** | 2 ml/kg of 3% Tween 80 | 2.36±0.50$^{a}$ | |
| **2** | 10 mg/kg Pentazocine | 4.40±0.81$^{d}$ | 46.36 |
| **3** | 100 mg/kg Extract | 3.13±1.21$^{b}$ | 24.60 |
| **4** | 200 mg/kg Extract | 3.27±0.88$^{b}$ | 27.83 |
| **5** | 400 mg/kg Extract | 3.88±1.23$^{c}$ | 39.18 |

The values are shown as mean ± SD, with n = 4.
Mean values containing different alphabetic letters in the column exhibit a significant difference ($p < 0.05$).

### Discussion

Acetic acid-induced writhing in rats has been ascribed to intestinal discomfort and has attracted more attention in the investigation of medications for pain relief (Hasan *et al.*, 2010). By inducing a local inflammatory response, it is a technique to evaluate pain perception and peripherally acting analgesics (Hassan *et al.*, 2008). Tissue phospholipids release free arachidonic acid in response to pain stimulation (Ribeiro *et al.*, 2000; Franzotti *et al.*, 2002). Peritoneal mast cells (Woiley, 2004), acid-sensing ion channels (Hossain *et al.*, 2006), and prostaglandin pathways (Adzu *et al.*, 2003) are thought to be involved in mediating the response. Another theory suggests that acetic acid exerts its indirect effects by bringing the issue of endogenous mediators from nociceptive neurons that are delicate to NSAIDs and narcotics (Alam *et al.*, 2012). Rats' acetic acid-induced writhes and wrinkles were significantly ($p<0.05$) and dose-dependently reduced by the S. magnificum extract. The extract's effect at 400 mg/kg was equivalent to 100 mg/kg of aspirin. This finding raises the possibility that the extract possesses peripherally mediated NSAID-like analgesic properties. NSAIDs have the ability to inhibit COX in peripheral tissues, which can disrupt primary afferent nociceptors' transduction mechanism. Therefore, similar to aspirin's peripherally mediated mechanism of action, the analgesic action of S. magnificum extract may be due to inhibition of the effect or release of endogenous substances that stimulate pain nerve endings, such as bradykinin. The result obtained is consistent with the previous report of Ilodigwe and Akah (2009) and Alam *et al.* (2013) who observed a decrease in the number of acetic acid-induced writhes in mice by *Spathodea campanulata* and Piper betle leaf extracts, respectively. The hot plate model is a well-established method for identifying the opiate (narcotic) analgesic properties of centrally acting analgesics. It is based on nociceptive responses to thermal stimuli (Kumari *et al.*, 2013). In the hot plate test, S. magnificum extract established a significant ($p < 0.05$) dose-dependent analgesic effect, involving expanded brain functions and supraspinal responses to nociceptive stimuli. (Mohammed *et al.*, 2014). Thus, the outcome might imply that the extract also has a central analgesic influence. This pain-relieving effect of *S. magnificum* extract can be partially accredited to its anti-inflammatory effect, as in the intestinal pain model, the release of arachidonic acid via precursor cyclooxygenase and prostaglandin biosynthesis as reported by the previous study (Joshua *et al.*, 2020) which plays a part in the nociceptive mechanism (Franzotti *et al.*, 2002). This indicates that the extracts' suppression of acute inflammation has a suppressive effect on the onset of pain. Ilodigwe and Akah (2009) also reported an increase in latency time to thermal pain by *Spathodea campanulata* leaf extract. The extract, however, seems to have been more successful in reducing central pain than peripheral (acetic acid) pain, based on the analgesic results of the acetic-induced writhing response and thermal experiments. According to Omeh and Ezeja (2010), the tail-flick response is a model of deep pain that is believed to be a spinally mediated reflex. The effectiveness of analgesic medications in this model is strongly associated with reducing

human pain perception. Based on the finding that morphine-like substances can precisely lengthen the time it takes for rats to experience the typical tail-flick withdrawal effect, the tail-flick method was developed (Raghunatha *et al.*, 2017). The screening process is frequently employed to determine how extracts affect the central nervous system (CNS). When compared to rats treated with Tween 80, the extract significantly ($p<0.05$) increased the PRT of treated mice to thermal stimuli in a dose-dependent manner. This specifies that the extract might raise the rats' CNS pain threshold. When compared to the effects of pentazocine (10 mg/kg), the extract's CNS involvement was less pronounced. According to Rang *et al.* (2007), pentazocine is a synthetic mixed agonist-antagonist that has morphine-like analgesic properties. The result of anti-nociceptive effects of S. magnificum extract is in pact with the report of Prabhavathi *et al.* (2012) who observed significant ($p<0.05$) rise in the mean reaction time of rats treated with gentamicin. Interestingly, compounds found in the extract, such as flavonoids (Joshua *et al.*, 2020) and partly triterpenes, have been shown to have analgesic activity as claimed by Pritam *et al.* (2011) and Okwu and Josiah (2006). Tannins' ability to scavenge free radicals and inhibit iNOS in macrophages may have an impact on the inflammatory response (Jeffers, 2006). Conversely, saponins use NO inhibition to block pain and inflammation (Moharram and Al-Shenawi, 2007, Hassan *et al.*, 2012).

## CONCLUSION

The outcomes of this investigation demonstrated the efficacy of *S. magnificum* extract in reducing pain that was induced by heat as well as chemically (inflammatory). This potential may be credited to the primary metabolites existing in the plant as reported in our previous work. It is suggested here that *S. magnificum* be used as a substitute medicinal plant extract to treat acute pain. To identify the active ingredient or ingredients that give *S. magnificum* leaf extract its analgesic effects and to understand its mode of action, more research is required.

## AUTHORS' CONTRIBUTIONS

Writing - original draft preparation, Chizoba Joy Anosike;, Investigation - Chizoba Joy Anosike Jachike Roland Aneke and Emenike Benjamin Amadi.; Writing - review and editing, Chizoba Joy Anosike and Rita Onyekachukwu Asomadu.; Supervision - Parker Elijah Joshua.; Funding acquisition - Chizoba Joy Anosike.; All authors have read and agreed to the published version of the manuscript.

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## CONFLICT OF INTEREST

The author(s) herein state that they have no opposing interests with reference to the publication of this work.

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## REFERENCES

Adzu, B., Amos, S., Kapu, S.D. and Gamaniel, K.S. (2003). Anti-inflammatory and anti-nociceptive effects of Sphaeranthus senegalensis. *Journal of Ethnopharmacology*, 84(3): 169-173.

Alam, B., Akter, F., Parvin, N., SharminPia, R., Akter, S. and Chowdhury, J. (2013). Antioxidant, analgesic and anti-inflammatory activities of the methanolic extract of Piper betle leaves. *Avicenna Journal of Phytomedicine*, 3(2): 112-125.

Alam, B., Hossain, S., Habib, R., Ria, J. and Islam, A. (2012). Antioxidant and analgesic activities of *Lannea coromandelica* Linn. bark extract. *International Journal of Pharmacology*, 8: 224-233.

Bahmani, M., Shirzad, H., Majlesi, M., Shahinfard, N., & Rafieian-Kopaei, M. (2014). A review study on analgesic applications of Iranian medicinal plants *Asian Pacific Journal of Tropical Medicine*, 7(1): 43–53. https://doi.org/10.1016/S1995-7645(14)60202-9

Baradaran, A., Madihi, Y., Merrikhi, A., Rafieian-Kopaei, M. and Nasri, H. (2013). Serum lipoprotein (a) in diabetic patients with various renal function not yet on dialysis. *Pakistan Journal of Medical Sciences*, 29(1 SUPPL.). https://doi.org/10.12669/pjms.291(Suppl).3533

Dash, P.R., Rana, M. S. and Ali, M.S. (2015). "Investigation of analgesic and cytotoxic activities of ethanol extract of *Commelina appendiculata*," *Journal of Pharmacognosy and Phytochemistry*, 4: 53–59.

Franzotti, E.M., Santos, C.V.F., Rodrigues, R.H.V., Mourao, M.R. and Antoniolli, A.R. (2002). Anti-inflammatory, analgesic and acute toxicity of *Sida cardiafolia* L. *Journal of Ethnopharmacology*, 72: 273-278.

Harchaoui, L., Chabane, D. & Ouaf, S. (2018). Analgesic and anti-inflammatory properties of aqueous extract of *Deverra scoparia* Coss and Dur obtained from Tamanrasset, *Algeria. Tropical Journal of Pharmaceutical Research*, 17 (8): 1523-1529

Hasan, S.M.R., Hossain, M.M., Akter, R., Jamila, M., Mazumder, M.E.H. and Alam, M.A. (2010). Analgesic activity of the different fractions of the aerial parts of *Commelin abenghalensis* Linn. *International Journal of Pharmacology*, 6(1): 63-67.

Hassan, H.S., Ahmadu, A.A. and Hassan, A.S. (2008). Analgesic and anti-inflammatory activities of *Asparagus africanus* root extract. African *Journal of Traditional, Complementary and alternative medicine*, 5(1): 27-31.

Hassan, H.S., Sule, M.I., Musa, A.M., Musa, K.Y., Abubakar, M.S. and Hassan, A.S. (2012). "Anti-inflammatory activity of crude saponin extracts from five Nigerian medicinal plants," *African Journal of Traditional, Complementary, and Alternative Medicines*, 9: 250–255.

Hossain, M. M., Ali, M. S., Saha, A. and Alimuzzaman, M. (2006). Antinociceptive activity of whole plant extracts of *Paederia foetida*. *Dhaka University Journal of Pharmaceutical Science*, 5: 67-69.

Houghton, P.J., Osibogun, I.M. and Bansal, S. (1992). A peptide from *Schumanniophyton magnificum* with anti-cobra venom activity. *Planta Medica*, 58(3):263-265.

Ilodigwe, E.E. and Akah, P.A. (2009). *Spathodea campanulata*: an Experimental Evaluation of the Analgesic and Anti-inflammatory Properties of a Traditional Remedy. *Asian Journal of Medical Sciences*, 1(2): 35-38.

Jeffers, M.D. (2006). Tannins as Anti-Inflammatory Agents, Miami University.

Joshua, P.E., Anosike, C.J., Asomadu, R.O., Ekpo, D.E., Uhuo, E.N. and Nwodo, O.F.C. (2020). Bioassay-guided fractionation, phospholipase A2-inhibitory activity and structure elucidation of compounds from leaves of *Schumanniophyton magnificum*. *Pharmaceutical Biology*, 58(1): 1078–1085 https://doi.org/10.1080/13880209.2020.1839510

Koster, R., Anderson, M. and Debeer, E.J. (1959). Acetic acid analgesic screening. *Federation Proceedings*, 18: 418-420.

Kumari, A., Rao, J. and Kumari J. (2013). "Analgesic activity of aqueous extract of *Citrullus lanatus* peels," *Advances in Pharmacology and Pharmacy*, 1: 135–138.

Manglik, A., Lin, H., Aryal, D.K., McCorvy, J.D., Dengler, D. and Corder, G. (2016). Structure-based discovery of opioid analgesics with reduced side effects. *Nature*, 537(7619): 185–190. https://doi.org/10.1038/nature19112

Mohammed, M.S., Mohammed, M., Vinny, N., Mohammad, T. and Jacobus, N.E. (2014). Antiinflammatory and antinociceptive activities of Loxostylis alata A. Spreng. Exrchb (Anacardiaceae). *American Journal of Research Communication*, 2(7): 227-246.

Moharram, F.A. and El-Shenawy, S.M. (2007). "Antinociceptive and anti-inflammatory steroidal saponins from *Dracaena ombet*. *Planta Medica*, 73(10): 1101–1106.

Neto, A.G., Costa, J.M.L.C. and Belati, C.C., 2005. "Analgesic and anti-inflammatory activity of a crude root extract of Pfaffia glomerata (Spreng) Pedersen," *Journal of Ethnopharmacology*, 96(2): 87–91.

Okwu, D.E. and Josiah, C. (2006). Evaluation of the chemical composition of two Nigerian Medicinal plants. *African Journal of Biotechnology*, 5(4): 357-361.

Omeh, Y.S. and Ezeja, M.I. (2010). Analgesic activity of the methanolic leaf extract of *Jatropha Curcas* (Linn). *African Journal of Biomedical Research*, 13: 149-152.

Pelletier, J.P., Martel-Pelletier, J., Rannou, F. and Cooper, C. (2016). Efficacy and safety of oral NSAIDs and analgesics in the management of osteoarthritis: Evidence from real-life setting trials and surveys. *Seminars in Arthritis and Rheumatism*, 45(4), 22-27. https://doi.org/10.1016/j.semarthrit.2015.11.009

Raghunatha, R.P., Bhanu, P.K. and Raghunandan, M. (2017). Evaluation of analgesic effect of Gentamicin in thermally induced pain models in rats and mice. *National Journal of Physiology, Pharmacy and Pharmacology*, 7(3), 240-246. doi:10.5455/njppp.2017.7.0821723092016

Prabhavathi, N.B., Kowsalya, B., Kumar, R.S., Sravani, J.B., Sri, D.G. and Sakila, A. (2012). Analgesic activity of different solvent extract of *Operculina turpethum* by using swiss albino mice. *Asian Journal Pharmacy and Clinical Research*, 5(3): 215-218.

Pritam, S.J., Amol, T., Sanjay, B.B. and Sanjay, J.S. (2011). Analgesic activity of *Abelmoschu smonihot* Extracts. *International Journal of Pharmacology*, 7: 716-720.

Raghunatha, R.P., Bhanu, P.K. and Raghunandan, M. (2017). Evaluation of analgesic effect of Gentamicin in thermally induced pain models in rats and mice. *National Journal of Physiology, Pharmacy and Pharmacology*, 7(3): 240-246. doi:10.5455/njppp.2017.7.0821723092016

Rang, H.P., Dale, M.M., Ritter, J.M. and Moore, R.K. (2007). Pharmacology. 6th Edition. Edinburgh: Churchill Livingstone; p. 203-215.

Ribeiro, R.A., Vale, M.L., Thomazzi, S.M., Paschoalato, A.B., Poole, S. and Ferreira, S.H. (2000). Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. *European Journal of Pharmacology*, 387, 111-118.

Shreedhara, C.S., Vaidya, V.P., Vagdevi, H.M., Latha, K.P., Muralikrishna, K.S. and Krupanidhi, A.M. (2009). "Screening of *Bauhinia purpurea* Linn. for analgesic and anti-inflammatory activities. *Indian Journal of Pharmacology*, 41(2): 75–79.

Taber, R.I., Greenhouse, D.D., Rendel, J.K. and Irwin, S. (1969). Agonist and antagonist interactions of opiods on acetic acid induced abdominal stretching in mice. *Journal of Pharmacology and Exponential Therapy*, 169, 29-37.

Turner, R.A. (1965). Screening Methods in Pharmacology. Academic Press; New York. p. 158-164.

Voilley, N. (2004). Acid-sensing ion channels (ASICs): New targets for the analgesic effects of Non-Steroid Anti-Inflammatory Drugs (NSAIDs). *Current Drug Targets Inflammatory Allergy*, 3(1): 71-79.