

JACK FRUIT: An Estimation of *Hunteria Umbellata* Fruit Extract against Formalin Induced Nociception

Abstract

The purpose of the study is to ascertain the impact of ethanolic fruit extract from *Hunteria umbellata* on formalin-induced nociception in Wistar rats. Six sets of thirty Wistar rats, weighing 110.7 to 169.4 g each, were employed for the investigation. Group 1 functioned as the control group and was given nothing except unlimited amounts of rat food and water. Formalin was administered just to Group 2, which served as the FOG. Group 3 rats were given the standard drug group (SDG). Group 4 rats received 50 mg of *Hunteria umbellata* fruit extract per kilogramme of body weight. Group 5 rats received 100 mg of *Hunteria umbellata* fruit extract per kilogramme of body weight. Group 6 rats received 150 mg of *Hunteria umbellata* fruit extract per kilogramme of body weight. The experimental rats were given 50L of a 2% formaldehyde solution subcutaneously into their left hind paws an hour after extract and medication administration in order to cause pain. The duration of paw licking was immediately used to identify the spontaneous nociceptive behaviour. Following a 14-day period of treatment, samples were taken and tested for signs of pain. The significance level was set at $p < 0.05$ after post hoc LSD and SPSS analysis of the data. The outcome showed that, in contrast to the formalin only group, the extract (LDEG, MDEG, HDEG) significantly enhanced ($p < 0.05$) the reaction time of nociceptive behaviours (FOG). In contrast to the control group, serum nociceptive molecules considerably ($p < 0.05$) increased in the formalin only group (FOG). In conclusion, the extract proved useful for treating nociception by traditional medical professionals (herbalists).

Keywords: *Hunteria umbellata*, Jack Fruit, Nociception, Formaldehyde, Pain.

Introduction

Non-steroidal anti-inflammatory medicines (NSAIDs) are the most often prescribed medications for treating pain and inflammation in clinical settings (1, 23).

After reference 1 and 23, need to cite the following references:

Singh Y, Fuloria NK, Fuloria S, Subramaniyan V, Almalki WH, Al-Abbasi FA, Kazmi I, Rajput SS, Joshi N, Gupta G. A European pharmacotherapeutic agent roflumilast exploring integrated preclinical and clinical evidence for SARS CoV-2 mediated inflammation to organ damage. *Br J Clin Pharmacol.* 2022 Aug;88(8):3562-3565. doi: 10.1111/bcp.15328.

Bajaj S, Fuloria S, Subramaniyan V, Meenakshi DU, Wakode S, Kaur A, Bansal H, Manchanda S, Kumar S, Fuloria NK. Chemical Characterization and Anti-Inflammatory Activity of Phytoconstituents from *Swertia alata*. *Plants (Basel).* 2021;10(6):1109. doi: 10.3390/plants10061109.

Due to their poor potency and side effects, analgesic medications like opiates and NSAIDs are not thought to be helpful in every situation. The negative effects of opiates and NSAIDs could include opiate overdose, gastrointestinal problems, and liver failure (2). Finding better options is therefore necessary. Due to the presence of a varied and complex diversity of compounds from which it is possible to develop new analgesic drugs, medicinal plants can function as a viable alternative source (3).

After reference 3, need to cite the following reference:

Fuloria S, Mehta J, Chandel A, Sekar M, Rani NNIM, Begum MY, Subramaniyan V, Chidambaram K, Thangavelu L, Nordin R, Wu YS, Sathasivam KV, Lum PT, Meenakshi DU, Kumarasamy V, Azad AK, Fuloria NK. A Comprehensive Review on the Therapeutic Potential of *Curcuma longa* Linn. in Relation to its Major Active Constituent Curcumin. *Front Pharmacol.* 2022;13:820806. doi: 10.3389/fphar.2022.820806.

Hunteria umbellata, which is primarily found in Sub-Saharan Africa, is a helpful and beneficial medicinal plant having a wide range of domestic and ethnomedicinal uses. The plant is referred to

informally by the Edo, Igbo, and Yoruba people of Nigeria as "Osu", "Npokiri," "Abeere," and "Erin," respectively. It is known that *H. umbellata* has a wide range of traditional medical use in the area. In African traditional medicine (ATM), the plant's dry seeds are highly prized for treating a variety of human ailments. African traditional herbalists use various parts of the plant to treat veterinary and human conditions locally, including pain, gastric ulcers, liver conditions, obesity, diabetes mellitus, fever, leprosy sores, menstrual irregularities, infertility, yawning, intestinal worms, abdominal colic, and discomfort, to name a few (4).

After reference 4, need to cite the following reference:

Subramaniyan, Vetriselvan, Saminathan Kayarohanam, J AshokKumar and Vinoth Kumarasamy. "Impact of herbal drugs and its clinical application." *International Journal of Research in Pharmaceutical Sciences* (2019): n. pag.

Traditional midwives, for example, place a high importance on the cold water decoction prepared from the plant's fresh leaves in the induction and augmentation of labour, while the water decoction created from the mature and ripe fruits' seeds is used to cure infections, stomach ulcers, diabetes, and obesity. However, recent research has supported the traditional use of the fresh leaves as an oxytocic medication. Its oxytocic activity is also demonstrated to be mediated via muscarinic acetylcholinergic mechanism. *Hunteria zeylanica* stem bark alkaloids have also been reported to have antipyretic properties. Additionally, it has been discovered that an aqueous extract of the fruit pulp of *H. umbellata* can effectively control fever without affecting its antibacterial activities (5).

Additionally, *Hunteria umbellata* has been demonstrated to be highly effective against bacteria including *Escherichia coli*, *Proteus* spp., and *Staphylococcus aureus* (6). Recent studies have shown that the aqueous seed extract of *H. umbellata* has oral hypoglycaemic activity of 50–200 mg/kg in a variety of hyperglycemic models, which was mediated by inhibition of intestinal glucose uptake, inhibition of adrenergic mechanism, increased peripheral glucose uptake, and improvements in insulin resistance (6).

Without any scientific backing, herbalists in Nigeria use this plant to dull the perception of pain.

The current study was fashioned to assess the analgesic ability of the ethanol fruit extract of *H. umbellata* in Wistar rat.

Materials & Methods

Experimental Animals

A total of thirty (30) Wistar rats were randomly allocated into distinct groups. They ranged in weight from 110.7 to 169.4 grammes. The rats in each group were housed in separate cages at Chukwuemeka Odumegwu Ojukwu University's animal house, where they were exposed to natural light and dark cycles and given two (2) weeks of acclimatization as well as unlimited access to normal rat food and water ad libitum. The National Institute of Health Guidelines for the Care and Use of Laboratory Animals were followed for each rat (7).

Extract preparation

The Fresh fruits were obtained from the market, and were washed thoroughly using distilled water. The fruits were placed on a fenestrated mat to allow the water to drain and air dried for about 28 days. After drying, the fruits were weighed before and after grinding into powdered form and were stored in airtight containers, and kept in a clean, dry, damp free store at normal room temperature till they're ready to be used.

LD₅₀ determination

The median lethal dose (LD₅₀) of *Hunteria umbellata* fruit was carried out in the department of Physiology, Faculty of Basic Medical Science, Chukwuemeka Odumegwu Ojukwu University, Uli campus. This was determined using a method of Dietrich Lorke (8).

Experimental Animal Groupings

The animals were randomly divided into six (6) groups of five (5) animals each.

Group 1 served as the control group and received only rat feed and water ad libitum.

Group 2 served as the formalin only group (FOG) and received formalin only
Group 3 rats were the standard drug group (SDG) and received 100mg/body weight of diclofenac 14 days

Group 4 rats were the low dose extract group (LDEG) and received 50mg/body weight of *Hunteriaumbellata* fruit extract 14 days.

Group 5 rats were the medium dose extract group (MDEG) and received 100mg/body weight of *Hunteriaumbellata* fruit extract for 14 days.

Group 6 rats were the High dose extract group (HDEG) and received 150mg/body weight of *Hunteriaumbellata* fruit extract for 14 days.

Formaldehyde Induced Pain Model and Nociceptive Behaviour

After one hour of the administration of extract and drug, 50 μ L of 2%formaldehyde solution was injected subcutaneously into the left hind paw of the experimental rats. The spontaneous nociceptive behaviour was immediately determined by measuring the duration of paw licking (9, 10). Rats were immediately placed in a transparent plastic cage and the paw licking time and frequency was recorded from initial 0 to 5 min (first-phase, neurogenic) and then 15 to 30 min (second-phase, inflammatory).

The percent (%) inhibition of the duration of licking was also calculated as follows:

Inhibition (%) = $\frac{\text{Duration of paw licking (control)} - \text{Duration of paw licking (test)}}{\text{Duration of paw licking (control)}} \times 100$

Sample collection

At the end of the fourteen days treatment with *H. umbellata*, animals were anesthetized using chloroform in an enclosed container for two minutes. After 2 minutes, blood samples were collected from the animals using a capillary tube through ocular puncture. Blood obtained were put in a well-labelled EDTA container and were centrifuged at 1000RPM to obtain serum for two minutes.

Determination of Pain Molecules

Serum pain chemicals were analyzed by enzyme linked immunosorbent assay (ELISA).

Statistical Analysis of Results

Data obtained from this study was analyzed using Statistical Science for Social Sciences (SPSS) version 25. Data obtained for brain weight was analyzed using ANOVA followed by post hoc LSD. Data was considered significant at $p < 0.05$.

Results

Figure 1a: Body weight values of extract in study animals

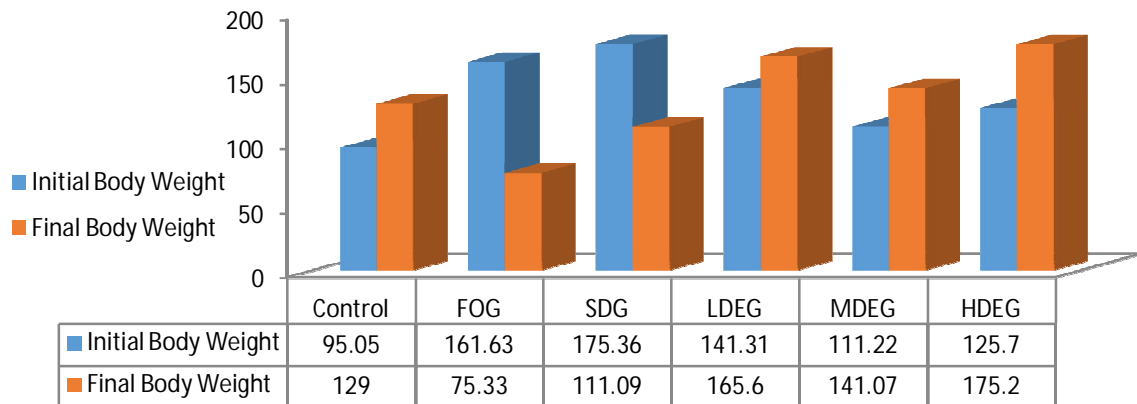


Figure 1b: % Diff of initial and final body weight in study animals

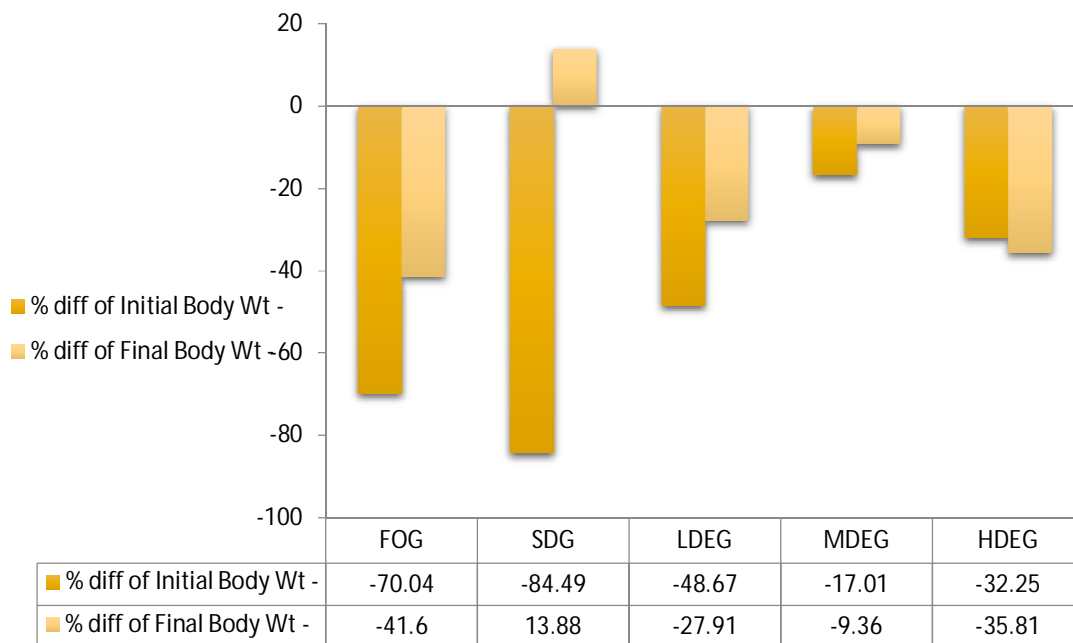


Figure 2a: Pain behavioral values of study animals after 7 days of extract administration

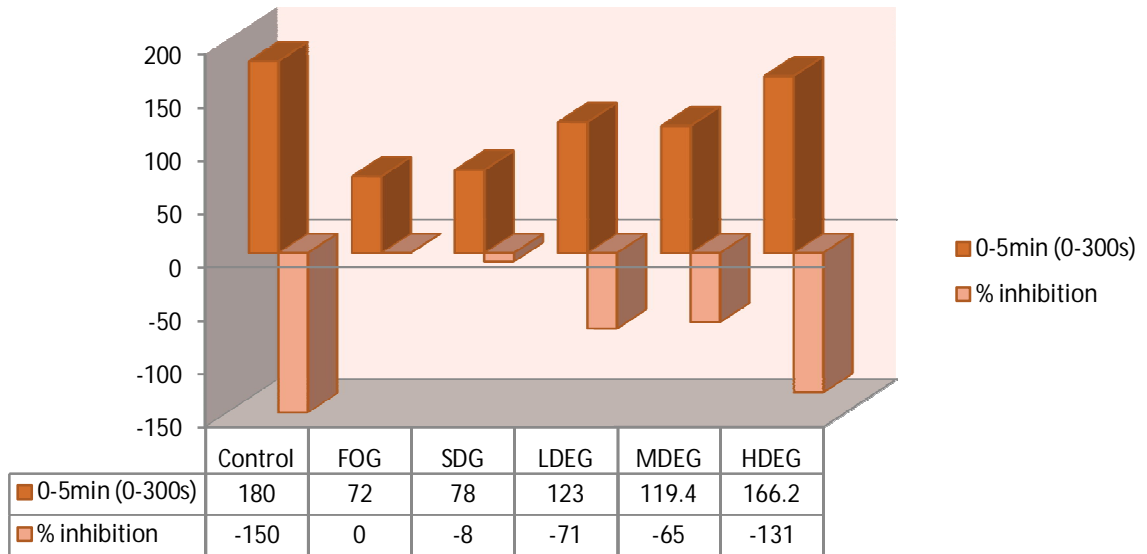


Figure 2b: Pain behavioral values of study animals after 14 days of extract administration

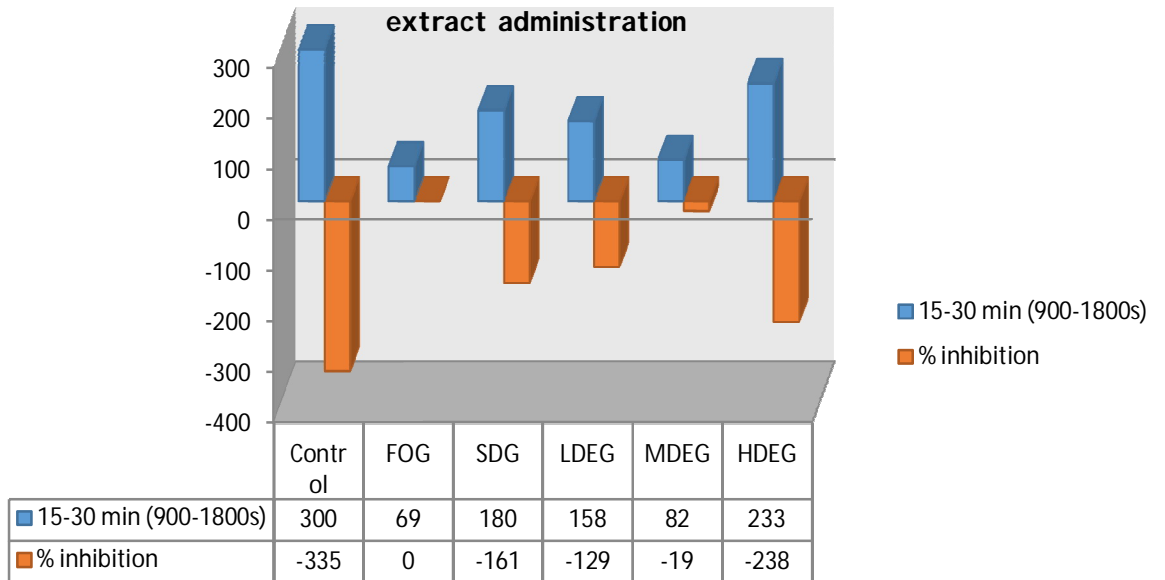
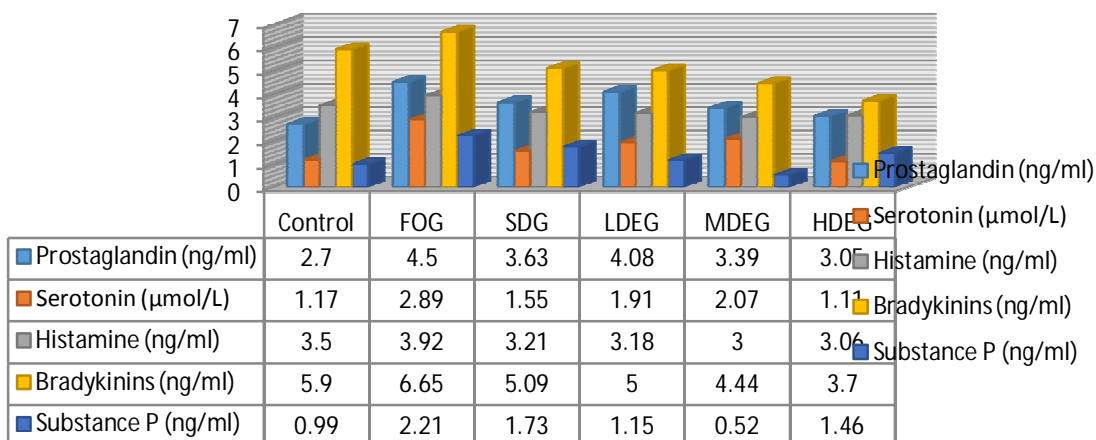


Figure 3: Values of pain chemicals of extract in study animals



Discussion of findings.

Pain is a distressing sensory and affective experience that is connected to or defined in terms of tissue damage, whether it is present or potential. Pain is characterised as a subjective, unpleasant, physical, and psychological sensation that is felt when specific nerve fibres in the spinal cord are stimulated and travel along specific pathways to the brain (11).

After reference 11, need to cite the following references:

Chinnasamy V, Subramaniyan V, Chandiran S, Kayarohanam S, Kannian DC, Velaga VSSR, Muhammad S. Antiarthritic Activity of *Achyranthes Aspera* on Formaldehyde - Induced Arthritis in Rats. *Open Access Maced J Med Sci.* 2019;7(17):2709-2714. doi: 10.3889/oamjms.2019.559.

Subramaniyan V, Fuloria S, Gupta G, Kumar DH, Sekar M, Sathasivam KV, Sudhakar K, Alharbi KS, Al-Malki WH, Afzal O, Kazmi I, Al-Abbasi FA, Altamimi ASA, Fuloria NK. A review on epidermal growth factor receptor's role in breast and non-small cell lung cancer. *Chem Biol Interact.* 2022;351:109735. doi: 10.1016/j.cbi.2021.109735.

The term "pain" refers to both nociceptive pain, which is frequently caused by tissue damage that activates nociceptive receptors, and non-nociceptive pain, which can result from injury to neural structures (neuropathic pain or neuralgia). While the latter is highly difficult to treat, there may or may not be signs of injury, causes chronic pain, and will linger long after the initial injury has healed the former is frequently acute, self-limiting after healing, and responds well to analgesics (12).

After reference 12, need to cite the following references:

Mohd Zaid NA, Sekar M, Bonam SR, Gan SH, Lum PT, Begum MY, Mat Rani NNI, Vaijanathappa J, Wu YS, Subramaniyan V, Fuloria NK, Fuloria S. Promising Natural Products in New Drug Design, Development, and Therapy for Skin Disorders: An Overview of Scientific Evidence and Understanding Their Mechanism of Action. *Drug Des Devel Ther.*

2022 Jan 6;16:23-66. doi: 10.2147/DDDT.S326332. PMID: 35027818; PMCID: PMC8749048.

Venkateshan S, Subramaniyan V, Chinnasamy V, Chandiran S. Anti-oxidant and anti-hyperlipidemic activity of *Hemidesmus indicus* in rats fed with high-fat diet. *Avicenna J Phytomed.* 2016;6(5):516-525.

The current study used the formalin-induced pain method to examine the anti-nociceptive properties of the ethanol fruit extract of *H. umbellata*.

As an experimental model of analgesia, formalin-induced pain is beneficial for illuminating the mechanisms behind both pain and analgesia because it evaluates the body's reaction to a persistent nociceptive stimulation and, as a result, is similar to clinical pain (13).

After reference 13, need to cite the following reference:

Singh Y, Fuloria NK, Fuloria S, Subramaniyan V, Almalki WH, Gupta G, Shaikh MAJ, Singh M, Al-Abbasi FA, Kazmi I. Disruption of the biological activity of protease-activated receptors2/4 in adults rather than children in SARS CoV-2 virus-mediated mortality in COVID-19 infection. *Drug Dev Res.* 202;82(8):1075-1078. doi: 10.1002/ddr.21874.

A biphasic nociceptive response is produced when rats' hind paws are subcutaneously injected with dilute formalin. Formalin directly affects sensory C-fibers, causing the first transient phase. The second prolonged phase is linked to the development of injury-induced spinal sensitisation, which is responsible for facilitating pain processing. Inflammatory pain also causes a central sensitisation of the dorsal horn neuron (1, 23). Medicines with a central mechanism of action, such as opioids, block both stages of formalin-induced pain, whereas drugs with a peripheral mechanism of action, such as diclofenac, block only the late phase (1, 23).

After references 1 and 23, need to cite the following reference:

Malik M, Bhatt P, Kumar T, Singh J, Kumar V, Faruk A, Fuloria S, Fuloria N, Subramaniyan V, Kumar S. Significance of chemically derivatized starch as drug carrier in developing novel drug delivery devices. *The Natural Products Journal.* 2022;12.

The current study's findings demonstrated that, when compared to the control and extract groups, the reaction time in the formalin-only treated rats (group 2) dramatically decreased. Reduced pain behaviour reaction times are a sign that an animal is experiencing extreme or increased pain (14).

However, the extract greatly lengthens the time it takes for pain to react, inhibiting both the early and late phases of formalin-induced pain, indicating both its central and peripheral anti-nociceptive activities. Aside this, HU produced more inhibition of the early phase than the late phase and was more efficacious than the conventional medication, diclofenac, in this phase.

The current study's findings also showed that group 2 (FOG), as compared to the extract groups, had much higher levels of the pain compounds typically generated by wounded tissues.

Substance P participates in the early phase of the formalin test, whereas histamine, serotonin, and prostaglandins are implicated in the late phase, and bradykinin affects both phases (15, 16, 17, 20).

After references 15, 16, 17 and 20, need to cite the following reference:

Safi SZ, Saeed L, Shah H, Latif Z, Ali A, Imran M, Muhammad N, Emran TB, Subramaniyan V, Ismail ISB. Mechanisms of β -adrenergic receptors agonists in mediating pro and anti-apoptotic pathways in hyperglycemic Müller cells. *Mol Biol Rep.* 2022;49(10):9473-9480. doi: 10.1007/s11033-022-07816-0.

Formalin-induced muscular contraction is a sensitive method to identify peripherally acting analgesics, and it is believed that such a reaction involves local peritoneal receptors. By reversing or lowering the heightened pain molecules generated by formalin, significant protection was seen in the extract-treated groups of rats (LDEG, MDEG, and HDEG), and this compared favourably with the usual medication. Diclofenac is one of the medications that is most usually used to treat mild to moderate pain, including the pain associated with migraines and fever. In the management of moderate to severe pain, it is frequently used with other non-steroidal anti-inflammatory medications and opioid analgesics (18). Although the exact mechanism by which diclofenac inhibits prostaglandin formation is complicated, its effect results in analgesia (19).

After reference 19 need to cite the following reference:

Fuloria S, Subramaniyan V, Karupiah S, Kumari U, Sathasivam K, Meenakshi DU, Wu YS, Guad RM, Udupa K, Fuloria NK. A Comprehensive Review on Source, Types, Effects, Nanotechnology, Detection, and Therapeutic Management of Reactive Carbonyl Species Associated with Various Chronic Diseases. *Antioxidants (Basel).* 2020;9(11):1075. doi: 10.3390/antiox9111075.

According to reports, HU contains secondary metabolites which include alkaloids, saponins, tannins, flavonoids, and glycosides (6). In several natural products, analgesic and anti-inflammatory effects have been linked to alkaloids.

Prostaglandins, which are implicated in the late stage of acute inflammation and pain perception, are known to be targeted by flavonoids (22). The involvement of tannins and saponins in anti-nociceptive and anti-inflammatory actions has also received little attention. In vitro histamine release inhibition by saponins has also been documented (21). Thus, HU's analgesic effect may have been caused by the presence of these biologically active components.

Conclusions

The ethanol fruit extract of *H. umbellata* showed remarkable anti-nociceptive properties in the animal models employed in this study, demonstrating its effectiveness in the management of pain by conventional medical practitioners (herbalists).

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