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Evaluation of the Analgesic Activity of Ethanolic Extract of *Manilkara Zapota* Seeds in Experimental Animal Model

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Non-Steroidal Anti-Inflammatory Drugs and opioids use remain the mainstay for pain treatment. However, both groups of drugs are well known for their common and severe side effects. Medicinal plants could be the better option to overcome these side effects. The anti-inflammatory activity of seeds of Manilkara zapota has already been demonstrated in experimental animals. As pain always accompanies inflammation, we have set forward a study to discover the analgesic activity of the ethanolic extract of Manilkara Zapota in animal models using Eddy's hot plate method. To explore the analgesic activity of ethanolic extract of Manilkara zapota seeds using Eddy's hot plate method. To compare the effect of ethanolic extract of Manilkara zapota seeds with the commonly used analgesic drug aspirin. This study was conducted by using Eddy's hot plate method. A total of 12 Adult Wistar albino rats were grouped into three groups of 4 animals in each group. These groups were Group I: Control (normal saline), Group II: Standard (Aspirin 25mg per kg) Group III: Ethanolic extract of Manilkara zapota (200mg per kg). The reaction time was recorded at 0, 30, 60 & 90 minutes after injecting normal saline, standard drug, and extract. The increase in mean reaction time of the extract was statistically significant ($p < 0.0001$) at 30, 60, and 90 min compared to that of the control. There was less increase in the reaction time of extract treated group compared to that of the aspirin-treated group at 30 min & 60 min points. The analgesic activity of the extract was significantly more than that of the control group. Peak analgesic activity occurred at 60 and 90 minutes.

Keywords: Analgesia; Eddy's hot plate method; Manilkara zapota seeds; Non-Steroidal Anti-Inflammatory Drugs.

The International Association for the Study of Pain (IASP) has defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage".[1] Pain could be more well-defined but almost associated with all medical conditions. It is often provoked by noxious stimuli, which can be of external or internal origin.[2] Pain obstructs daily activities as well as the general functioning of the body. The majority of the patients consult the physician because of the pain. It is the foremost & chief symptom of many diseases.

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Central and peripheral analgesics relieve pain without affecting consciousness. Analgesics are divided into opioids and Non-steroidal anti-inflammatory Drugs (NSAID). Opioids are indicated in deep-seated visceral pain, while NSAIDs are in pain associated with inflammation & tissue injury.[3] NSAID and opioids use remain the mainstay for the treatment of pain. However, both groups of drugs are well known for their common & severe side effects. The studies conclude that opioids lead to tolerance, physical dependence, and addiction, whereas NSAIDs lead to gastrointestinal bleeding & ulcers.[4]

For that, looking for an alternative compound with minimal or no side effects for pain management is necessary. Medicinal plants could be the better option to overcome these side effects.

Conventional drug therapy for diseases can occasionally have severe adverse effects. All over the world, there is a trend to explore traditional medicines. Siddha is an old medicine branch famous amongst the Tamil-speaking people. There are various sources of the drugs. These can be broadly classified into plants, animals, microorganisms & minerals. There is a need to identify the accurate source of the drug, which can give good results with minimal side effects. The availability of plant sources is more as compared to the other sources. The newly discovered active compound should have higher efficacy, safety, and fewer side effects.

Medicinal plants contain many chemical substances which have possible and multiple therapeutic uses. Medical medicinal plants have been used for therapeutic use in many disease conditions worldwide. Still, the active compounds from the medicinal plants are used to prepare drugs. The principal advantage of herbal medicine is that they are safer than synthetic medicine as they have fewer side effects and are cost-effective, which would be accepted by developing countries like India.

From ancient times, different parts of medicinal plants were in use to cure different diseases, especially in India.[5] Manilkara zapota belongs to the Sapotaceae family, which is commonly called by different names in different languages, such as Sapota in Hindi, American bully in English, Simaiyiluppai in Tamil, and Sapotasima in Telugu.

Manilkara zapota is a big tree with rough, lumpy shady grey bark and a thick crown. Leaves are elliptic-oblong, 7.5-12.5 cm long, shining from both sides with small, discrete secondary branching nerves. Flowers are lengthy. Fruits are round with 5-6 big seeds and light brown pulp. The seeds are hard and brown or black with one white margin. The seeds contain phytochemicals like alkaloids, flavonoids, saponins, tannins, steroids, glycosides, and phenolic compounds. Hydrocyanic acid is also present in seeds, so it should be removed before eating the fruit.[6]

Seeds of Manilkara zapota have been evidenced to have diuretic,[7] antibacterial,[8] hypoglycemic,[9] and anthelmintic activity.[10] Bark extract has been assessed for antimicrobial and anticancer activities.[11] The plant leaves hold analgesic and anti-inflammatory,[12] antioxidant, hypoglycemic, and hypocholesterolemic activities.[13] The roots of the plant are found to have hypoglycemic activity.[14]

Although many pharmacological investigations have been carried out based on the constituents present in it, a lot more can still be explored and utilized in a therapeutic manner.

The anti-inflammatory activity of seeds of Manilkara zapota has already been demonstrated in experimental animals.[15] As pain always accompanies inflammation, we have set forward a study to discover the analgesic activity of the ethanolic extract of Manilkara Zapota in animal models using Eddy's hot plate method.

Aims and Objectives

To explore the analgesic activity of ethanolic extract of Manilkara zapota seeds using Eddy's hot plate method.

To compare the effect of ethanolic extract of Manilkara zapota seeds with the commonly used analgesic drug aspirin.

MATERIAL AND METHODS

This study was conducted after the approval of the Institutional Ethics Committee (IEC) (IAEC/SSSMCRI/2018-05). The study followed the Helsinki Declaration. The seeds of Manilkara zapota were obtained from a forest near the experimental institute. These fresh seeds were thoroughly cleaned with water to remove dirt. Seeds were dried for one week in the shed. After

complete drying, the seeds were powdered. The powder was packed in a sealed bottle and stored in a dry, cool, and dark place.

The extract is prepared with the help of Soxhlet's apparatus (hot extraction) and extracted with 95% ethanol at a temperature of 60°-80°C. Ethanolic extract was subjected to filtration. It was then dried under reduced pressure to obtain a solid mass that was free from the solvent. The dried extract was dissolved in distilled water.

12 Adult Wistar albino rats of weight ranging from 235 to 255 g were selected for this study. Following their procurement, they were kept at 24°C temperature and 55–65 % humidity to acclimatize and were provided a pellet meal and water as needed. The animals were divided into the following three groups, each with four animals.

The animals were grouped into the following three groups of 4 animals in each group.
Group I: Control (normal saline),
Group II: Standard (Aspirin 25mg/kg)
Group III: Ethanolic extract of Manilkara zapota (200mg/kg)

Normal saline, Aspirin & Ethanolic extracts of Manilkara zapota were given orally to animals. The dose of Aspirin & Ethanolic extract of Manilkara zapota was calculated according to the weight of each animal.

This study was carried out by using Eddy's hot plate method. The individual animal was placed on a plate and maintained at a temperature of 55°C.[16] Following the administration of normal saline, standard medication, and extract, the reaction time was recorded at 0 min, 30 min, 60 min, and 90 minutes. Reaction time was defined as the time animals responded to the pain stimulus by jumping or licking their paws. The reaction time was set at 45 seconds to prevent harm to an animal's paw.[17]

Data were entered on a Microsoft Excel spreadsheet and statistically analyzed statically using the SPSS-20 software. Unpaired student t-tests were used to compare reaction times. A p-value of 0.05 or less than 0.05 is indicated as statistically significant.

OBSERVATIONS AND RESULTS

The mean reaction time of all three groups, i.e., control, standard & test groups, at all time points is illustrated in Table 1. Compared to animals treated with saline, the mean reaction

Table 1. Comparison of mean reaction time for all the three groups

Treatment	Mean reaction time (seconds)			
	0 minutes	30 minutes	60 minutes	90 min
Control	30.50	28.25	27.00	25.25
Standard	30.50	39.00	40.75	34.25
Test	30.25	36.25	38.50	38.50

Table 2. Analgesic effect of aspirin by hot plate method

Treatment	Mean reaction time (seconds)			
	0 minutes	30 minutes	60 minutes	90 min
Control	30.50	28.25	27.00	25.25
Standard	30.50	39.00 *	40.75 *	34.25 *

Table 3. Analgesic effect of Manilkara zapota seeds extract by hot plate method

Treatment	Mean reaction time (seconds)			
	0 min	30 min	60 min	90 min
Control	30.50	28.25	27.00	25.25
Test	30.25	36.25 *	38.50 *	38.50 *

time of the aspirin and extract-treated group was increased, as shown in Figure 1.

The mean reaction time for normal saline-treated animals was found to be decreased throughout 90 minutes. The mean reaction time for aspirin-treated & extract-treated animals was increased throughout 60 min, but after that, it was found to be declined. Compared to animals treated with saline, the reaction time was significantly increased in both group 2 and group 3. The result of this is depicted in Table 2, figure 2, table 3 & figure 3.

When the analgesic activity of aspirin and extract was compared, it was found that there was less increase in the reaction time of the group treated with extract group compared to that of the aspirin-treated group. The reaction time of the extract-treated group at 30 min was found to increase significantly compared with the aspirin-treated group. Whereas the mean reaction time of extract treated group at 60 min & 90 min was not found to be significantly increased in comparison with the standard, as shown in Table 4 & Figure 4

DISCUSSION

Recently developed allopathic drugs still exhibit mild to severe side effects. Various plants are still unique because of having fewer adverse effects. Therefore, the analgesic effect of plant products has been studied systematically.

As pain is the most common manifestation of most of the diseases. Many synthetic NSAIDs have been used as the primary analgesic, with common adverse side effects. Therefore, It is beneficial to investigate an alternative analgesic therapy.

Analgesics relieve pain selectively without affecting it by acting on the central or peripheral nervous system. Centrally-acting analgesics lower the pain threshold and alter the body's typical physiological reaction to pain. Conversely, peripherally acting analgesics prevent impulse generation at the chemoreceptor site for pain.[18]

Table 4. Comparison of Analgesic effect of Aspirin &ethanolic extract of Manilkara zapota seeds

Treatment	Mean reaction time (seconds)			
	0 minutes	30 minutes	60 minutes	90 minutes
Standard	30.50	39.00 #	40.75	34.25
Test	30.25	36.25 #	38.50	38.50

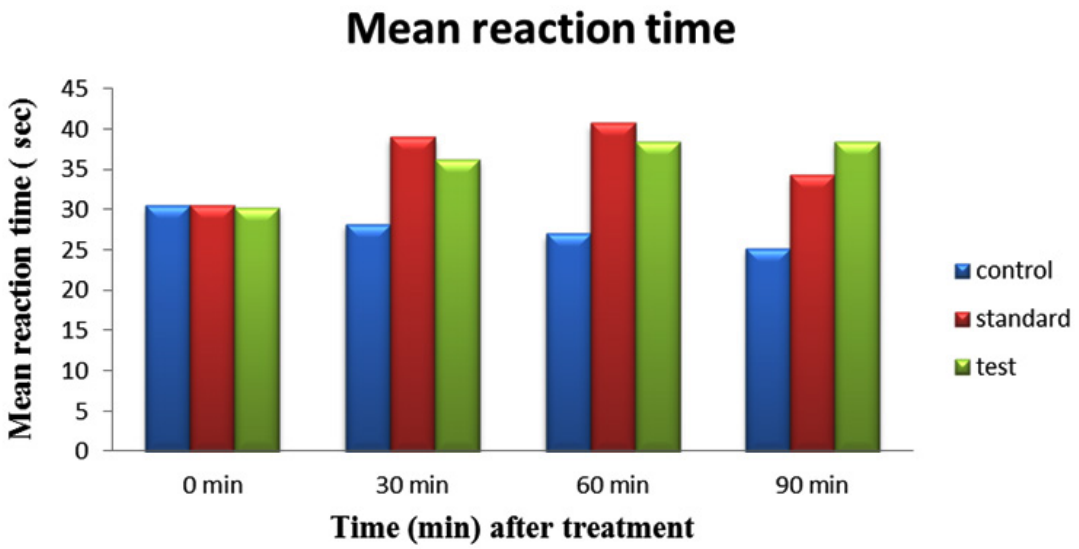

Fig. 1. Comparison of mean reaction time for all the three groups

Eddy's hot plate method is the animal model employed for screening analgesic activity in this study. In this experimental model for analgesia, an increase in reaction time is an important measure or parameter of central and peripheral analgesic activity.[19]

As mentioned earlier, many animal experiments have been conducted and proven many activities of Manikara Zapota, according to Ayesha Khan et al. The whole plant extract of Manilkara zapota Linn possesses analgesic activity. Therefore, in this study, particularly seeds of the Manilkara zapota plant were screened for analgesic effects by using Eddy's hot plate.

Manilkara zapota seeds extract showed a moderate to significant analgesic effect, which was proved by the significant increase in the reaction time, as depicted in Table 3 and Figure 3. Compared to the control, the extract's reaction time was similar at 0 min and slowly increased from 30, 60, and 90 min. The increase in mean reaction time of the extract was statistically significant (* $p < 0.0001$) at 30 min,60 min, and 90 min compared with that of the control. From this, it can be stated

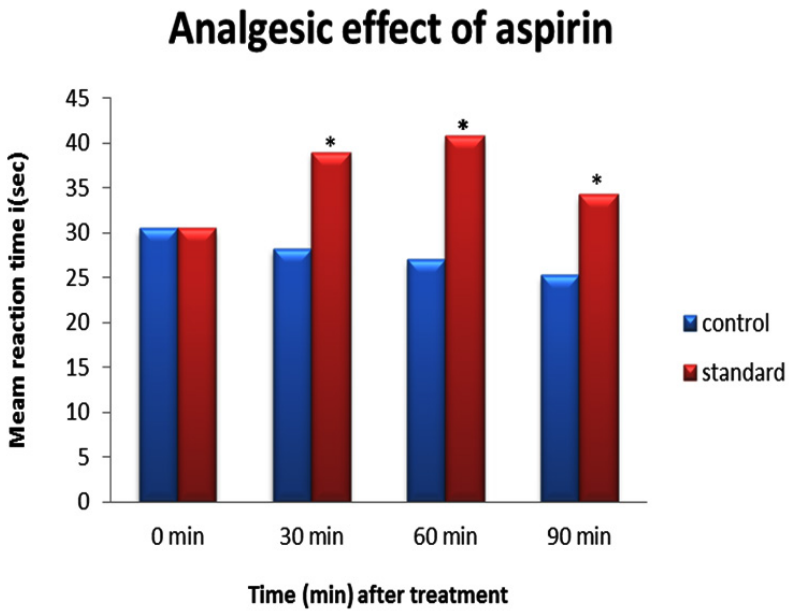

Fig. 2. Analgesic effect of aspirin by hot plate method

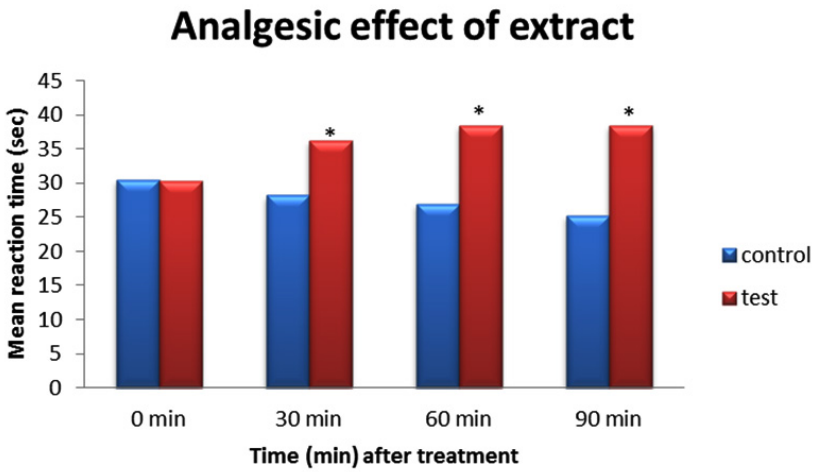

Fig. 3. Analgesic effect of Manilkara zapota seeds extract by hot plate method

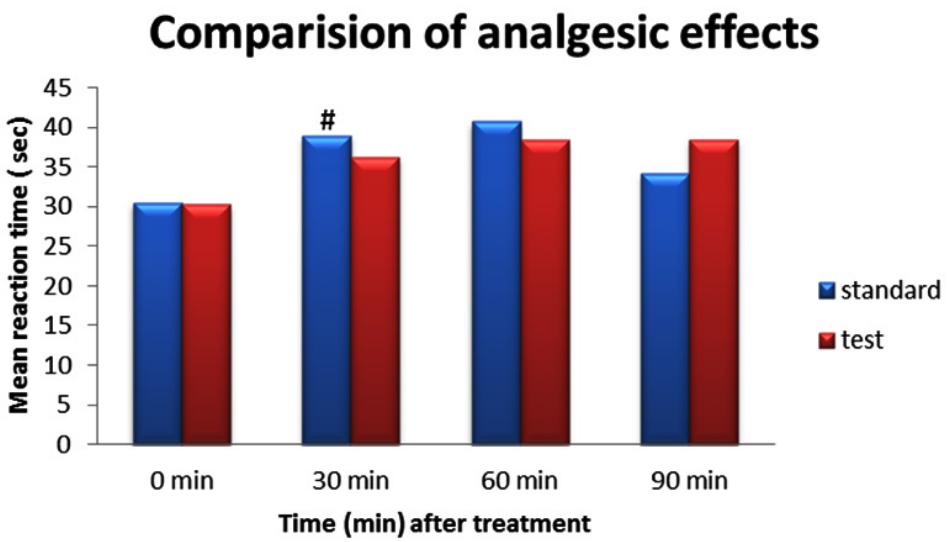

Fig. 4. Comparison of Analgesic effect of Aspirin &ethanolic extract of Manilkara zapota seeds

that the crude extract of Manilkara zapota seeds is an effective analgesic agent.

When the analgesic activity of aspirin and Manilkara zapota extract were compared, it was observed that there was less increase in the reaction time of the group treated with extract compared to that of the aspirin-treated group at 30 min & 60 min points of time. The Significant difference (# $p < 0.001$) in the analgesic activity of aspirin and the extract was found at 30 min. The analgesic effect of the extract-treated group at 90 min was found to be the same, but the reaction time for aspirin at 90 min was decreased compared to the earlier values, as shown in Table 4 & Figure 4. From this, we can state that the analgesic activity of aspirin has decreased earlier than that of the extract of seeds.

The observed analgesic effect of Manilkara zapota seed is attributed to the crude extract. Any crude extract contains many phytochemicals such as steroids, alkaloids, flavonoids, phenolic compounds, etc. Furthermore, the analgesic activity of seed extract may be attributed to the specific phytochemicals in the extract. A single constituent or more than one constituent may be responsible for the analgesic effect. Preliminary phytochemical screening of crude extract is needed. There is a need for additional studies to identify the actual phytochemicals in the crude extract of Manilkara zapota seeds, which are responsible for analgesic activity.

CONCLUSION

The extract under study had a significant analgesic effect compared with the analgesic activity of control in an established analgesic screening method. The analgesic effect was peaking at 60 minutes and 90 minutes. Treating 200mg/kg of ethanolic extract of Manilkara zapota seeds increases the reaction time on Eddy's hot plate. Hence, the present study concluded a significant analgesic effect of Manilkara zapota extract. Henceforth, the preparation of Manilkara zapota seed extract could be used as one of the drugs in multi-drug therapy for pain relief. Though, more research is needed in separating and describing the active compound or compounds responsible for the analgesic effect. Further work is also needed for the determination of the exact mechanism of action.

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REFERENCES

1. H.Merskey. Pain terms: a list with definitions and notes on usage. Recommended by the IASP Subcommittee on Taxonomy, Pain, 1979; 6: 249–252.

2. HL Sharma, KK Sharma. Principles of pharmacology.3rd edition.Paras Medical publisher
3. K. D. Tripathi, Essentials Of Medical Pharmacology, Jaypee Brothers Medical Publishers, New Delhi, India, 5th edition, 2004.
4. G. R. Hanson, P. J. Venturelli, and A. E. Fleckenstein, Drugs and Society, Jones and Bartlett, Boston, Mass, USA, 10th edition, 2009.
5. Bhattacharjee S.K. Handbook of Medicinal Plants. Pointer Publication Jaipur, India, 1998**.** p1-6.
6. Bano M, Ahmed B. Manilkara zapota (L.) P.Royen (Sapodilla): A Review. *Int J Adv Res Ideas Innov Technol*. 2017;3(6):2235-2240.
7. Shah B, Modi D, Desai R. Screening for Diuretic Potential of Manilkara zapota Seeds: Diuretic activity of Manilkara zapota seeds. Lambert Academic Publishing; 2012.
8. Kothari V, Seshadri S. In vitro antibacterial activity in seed extracts of Manilkara zapota, Anona squamosa, and Tamarindus indica. *Biol Res.* 2010;9:165-168.
9. Saradha AR, Ruckmini M, Chokkalingam R, Maingnakumar R. Hypoglycemic activity of aqueous and ethanolic extracts of Manilkara zapota seeds in streptozotocin induced diabetic rats. *Int J Pharm Pharm Sci*. 2014;6(2):434-437.
10. Yashvanthkumar DR, Vurivihema, Mayankagarval, Pramoditha Sruthy, Vedamurthy AB, Krishna V. Manilkara zapota seed embryo extract: a potent anthelmintic agent. *Asian J Pharm Clin Res.* 2012;5.
11. Osman MA, Aziz MA, Habib MR, Karim MR. Antimicrobial investigation on Manilkara zapota (L.) P. Royen. *Int J Drug Dev Res.* 2011;3(1):185-190.
12. Ganguly A, Al Mahmud Z, Nasir Uddin MM, Abdur Rahman SM. In-vivo anti-inflammatory and antipyretic activities of Manilkara zapota leaves in albino Wistar rats. *Asian Pac J Trop Dis*. 2013;3:301-307.
13. Fayek NM, Abdel Monem AR, Mossa MY, Meselhy MR, Shazly AH. Chemical and biological study of Manilkara zapota (L.) Van Royen leaves (Sapotaceae) cultivated in Egypt. *Pharmacognosy Res.* 2012;4(2):85-91.
14. Muhtadi, Gunawan, Abdulgani N, Gozali D. A study of antidiabetic activity of alcoholic extract of Achras zapota roots. *Bionatura* (Indonesia). 2000;2(2).
15. Khan A. Evaluation of analgesic and anti-inflammatory activity of whole plant extract of Manilkara zapota Linn. *World J Pharm Pharm Sci.* 2016;7:881-892.
16. Chatterjee C, Mandal G, Mukhopadhyay K, Das S, Mukherjee S, Chatterjee M, et al. Evaluation of analgesic activity of methanolic extract of Bougainvillea spectabilis leaves in experimental animal models. *Ann Int Med Dent Res*. 2016;2:1-4.
17. Fan SH, Ali NA, Basri DF. Evaluation of analgesic activity of the methanol extract from the galls of Quercus infectoria (Olivier) in rats. *Evid Based Complement Alternat Med*. 2014;2014:532692.
18. Shreedhara CS, Vaidya VP, Vagdevi HM, Latha KP, Muralikrishna KS, Krupanidhi AM. Screening of Bauhinia purpurea Linn. for analgesic and anti-inflammatory activities. *Indian J Pharmacol*. 2009;41:75-79.
19. Kitchen I, Crowder M. Assessment of the hot-plate antinociceptive test in mice: A new method for the statistical treatment of graded data. *J Pharmacol Methods*. 1985;13(1):1-7.