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IN VIVO EVALUATION OF ANTINOCICEPTIVE ACTIVITY FROM THE SEEDS OF MANILKARA ZAPOTA BY USING EDDY'S HOT PLATE METHOD.

Pharmacy

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ABSTRACT

The present study was to assess the Antinociceptive activity from the seeds of *Manilkara zapota* by using Eddy's hot plate method. The plant *Manilkara zapota* is an Sapotaceae family. In India *Manilkara zapota* seeds was used as various purposes and it contain following activities such as Anti-inflammatory, Analgesic, Antidiarrheal, Antifungal and Antibacterial activities. **Aim And Objective:** To evaluate Antinociceptive activity of seeds from *Manilkara zapota* and comparing to standard drug diclofenac. **Method:** Methonolic extract was prepared by using soxhlet apparatus. Take 4 groups of albino mice such as control, standard(diclofenac), 100mg and 200mg extract of seeds. **Result:** Comparing the 4 groups we found potent Antinociceptive activity from 100mg extract.

KEYWORDS

Manilkara zapota seeds, Antinociceptive activity, Eddy's hot plate, Albino mice.

INTRODUCTION:

Pain is the subjective experience, hard to define exactly, even though we all know what we mean by it. Typically it is a direct responses to an toward event associated with tissue damage such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cost or persist long after the precipitating injury has healed. It also occur an consequence of brain or nerve injury. Painful condition of the latter kind, not directly linked to tissue injury, or very common and major cause disability and distress, and in general they respond less well to conventional analgesic drugs than do conditions where the immediate cause is clear. In these cases, we need to think of pain in terms of disordered neural function, comparable with schizophrenia or epilepsy, rather than simply as a 'normal' responses to tissue injury[1].

Based on ethno medical information and review of literature *Manilkara zapota* seeds was selected for our investigation towards antinociceptive activity[2]. *Manilkara zapota* L. commonly known as sapodilla belongs to the family sapotaceae.

MATERIALS AND METHODS

Collection of seeds:

The seed of *Manilkara zapota* was collected from the cultivator's farm present within Kancheepuram, authenticated by **V.GANGADEVI, Ph.D.** Assistant Professor, PG Head and Research, Dept of Botany, Aringar Anna Govt Arts College, Cheyyar. The specimen No: AAGAC/BOT/005.

The seeds were collected in the month of September-2022. The collected seeds were cleaned to dust the adhering particle and dried under the shade. The dried material was coarsely powdered by means of mechanical grinder and the powder material was used for the extraction process.

Powder Microscopy

Fine dried powdered seed sample was separately treated with different solutions i.e. 50% glycerin, aqueous saturated chloral hydrate, phloroglucinol in conc.HCl and 0.02N iodine reagent, mounted on slides with 50% glycerin following a standard protocol and observed under the binocular compound microscope at 10x and 40x magnifications. The photomicrographs of different cellular structures and inclusion were taken using compound microscope.

Preparation of methanolic extract from the seeds of *Manilkara zapota:*

The seeds were shade dried for one month and coarsely powdered. Extraction of active ingredient from the seed powder was carried out using specific method. 60g of powdered seeds were extracted by Soxhlet apparatus using 500ml methanol in a separate flask. The extraction lasted for 12hrs. The extract obtained was concentrated by evaporation using electric water bath at 100°C and stored at 4°C in refrigerator[3],[4]. Then the percentage yield was calculated.

The obtained methanolic extract from the seeds of *Manilkara zapota* was subjected to preliminary Phytochemical studies and Pharmacological studies.**Preliminary Phytochemical screening**

The methanolic extract of *Manilkara zapota* was subjected to Preliminary Phytochemical screening for the qualitative detection of phytoconstituents such as alkaloids, steroids, triterpenoids, phenolic compounds and saponins[5].

Pharmacological studies

Animals:

Albino mice of either sex weighing between 20-30g with age of 8-10 weeks were procured from the animal house of Adhiparasakthi College of Pharmacy, Melmaruvathur. Standard housing was maintained and the animals were fed with a standard diet and with water *ad libitum* during the course of our study.

Dose Selection:

The dose selection was done on the basis of previous study. Diclofenac-10mg/kg Alcoholic extract of *Manilkara zapota* seeds 100mg/kg, 200mg/kg.

Procedure

Eddy's hot plate Method:

Hot plate was maintained at 55±0.2°C. In this method heat was used as a source of pain. Animals are individually placed on Eddy's hot plate and the reaction of animals such as paw licking or jump response was taken as the end point[6].

A total of 24 Swiss Albino mice were divided into 4 groups[7] (6 animals per group)
Group 1: Normal control
Group 2: Standard drug Diclofenac (10mg/kg)
Group 3: Alcoholic extract of *Manilkara zapota* seeds (100mg/kg)
Group 4: Alcoholic extract of *Manilkara zapota* seeds (200mg/kg)

All the animals were housed in clean polypropylene cages and to be maintained at standard condition of temperature (25±1°C) and 12:12

hour light/dark cycles. Standard pellet diet and free access to water to be provided.

The pre drug reaction time (PRT) will be accessed by placing each mice on Eddy's hot platemaintained at the temperature of about 55±0.2°C.

The PRT for each mice will be determined using a stopwatch to measure the time it took to lick the hind paw or jump above.The cutoff time was put at 15 secs. This served as a control reaction time[8],[9].
The hotplate test will be performed at 30, 60, 90 and 120 min after oral drug administration.

RESULTS AND DISCUSSION

Phytochemical Evaluation

Extractive value:

60g of powdered seeds were extracted by Soxhlet apparatus using 500ml methanol in a separate flask. The extraction lasted for 12 hours. The extract obtained was concentrated by evaporation using electric water bath at 100°C and stored at 4°C in refrigerator. Then the percentage yield was calculated.

Percentage yield = weight of crude drugs/ weight of powdered drug×100
=9.124/60×100
=15.20%w/w

Powder Microscopy:

Fine powder is oily, sticky granular mass with mixed black and cream coloured particles with stale oily taste and no characteristic odour, shows the presence of numerous sclereids of different shape and sizes, profuse oil globules throughout the sample, aseptate fibres and sclernchymatous cells containing oil globules.

DISCUSSION:

Antinociceptive effect of Manilkara zapota seeds persisted for a duration equally to that of Diclofenac Sodium. Diclofenac Sodium showed maximum effect at 90min and the effect lasted till 120min after administration where is methanolic extract of Manilkara zapota seeds(100mg/kg) showed maximum effect at 60min and the effect last beyond 120min after oral administration.

Comparatively the seed extract(100mg/kg) showed slightly more antinociceptive effect than the seed extract (200mg/kg). But there is no much difference in activity between 100 and 200mg. Dose dependent activity is not seen in seed extract. There may be some chemical constituents which is responsible for the suppression of activity at 200mgdose.

SUMMARY AND CONCLUSION

The methanolic extract of seeds of *Manilkara zapota* significantly exhibited antinociceptive activity.

This can be attributed to various phytochemicals such as alkaloids, triterpenoids, saponins, phenolic compounds present in the seed extract. In Eddy's hot plate method the extract significantly increased the reaction time when compared to the baseline. So the seed exhibit antinociceptive acivity in effective way. In conclusion *Manilkara zapota* seed extract can be developed a potent antinociceptive agent in future. More studies are needed to elucidate final decision about the particular chemical constituent which is responsible for the antinociceptiveactivity.

Figure 1: Shade dried and Methanolic extraction from the seeds of *Manilkara zapota.*

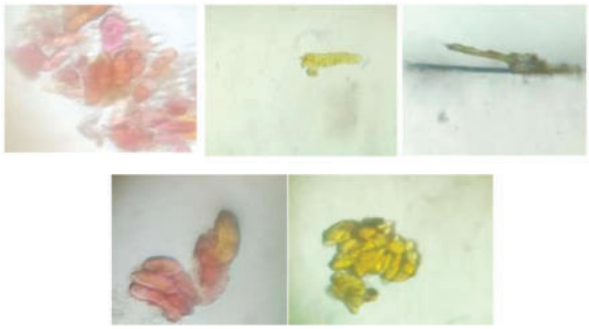

Figure 2: Powder microscopy of *Manilkara zapota* seeds

Figure 3: Jumping response and Paw licking response of mice

Chemical test:

Table 1: Identification of Phytoconstituents in *Manilkara zapota* seeds

S.NO	CHEMICAL TEST	OBSERVATION
1.	Detection of alkaloids	Positive
2.	Detection of triterpenoids	Positive
3.	Detection of phenolic compound and tannins	Positive
4.	Detection of saponins	Positive

Pharmacological Studies

Table 2: Invivo evaluation of Antinociceptive activity from the seeds of *Manilkara zapota* by using Eddy's Hot plate method.

Group	Mean Reaction Time				
	Pre-Drug	30 min	60min	90min	120min
Negative control	2.8±0.30	3±0.36	3.16±0.30	2.6±0.33	3±0.36
Diclofenac Sodium (10mg/kg)	3.8±0.31	6.7±0.20	9.3±0.23***	14.03±0.32***	8.6±0.56
Seed extract (100mg/kg)	3.66±0.33	9.5±1.54	14.16±0.47***	11.7±0.56***	10±0.36
Seed extract (200mg/kg)	3.8±0.36	10.5±0.76	13.27±0.83***	10.9±0.75***	9.25±0.60

Values are expressed in mean ± SEM, of six animals in each group (n=6). Analysis was performed with one way ANOVA followed by Dunnett's test.

The standard group, Extract-1 (100mg/kg) and Extract-2 (200mg/kg) were significantly different from control group at 60min and 90min.
*P<0.05, **P<0.01, ***P<0.001

Compared to Standard, Extract-1 (100mg/kg) produced quick analgesic effect at 60 mins. By comparing Extract-1 (100mg/kg) and Extract-2 (200mg/kg), the Extract-1 (100mg/kg) produce significant effect. ***P<0.001compared to control.

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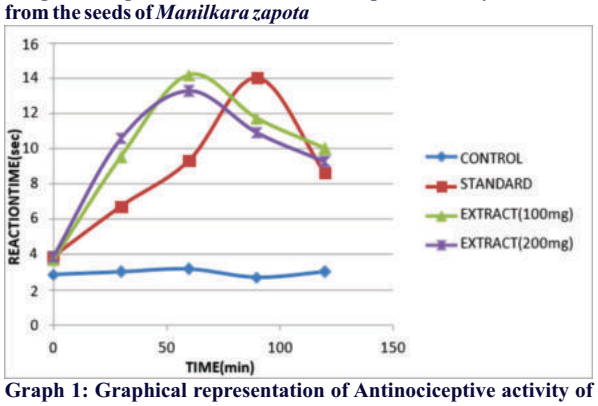
Graphical representation of Antinociceptive activity of extract from the seeds of *Manilkara zapota*



Graph 1: Graphical representation of Antinociceptive activity of extract from the seeds of *Manilkara zapota.*

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