Analgesic Activity of *Solanum melongena* Leaf Ethanolic Extract in Swiss Albino Mice by Writhing and Hot Plate Method

A Research Paper

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ABSTRACT

Solanum melongena Linn. is a herbaceous plant, with large felty leaves densely covered with tiny star-shaped (stellate) hairs, white to purple flowers, elongated or pear shaped fruit and are grown mainly for food and medicinal purpose. The aim of the study is to determine the analgesic activity of solanum melongena leaf ethanolic extract in swiss albino mice by acetic acid induced writhing method and hot plate method. For each method, albino mice were randomized and divided into 5 groups which received NSS, low dose(3.04gm/kg), middle dose(7.58gm/kg) and high dose leaf extract(19.05mg/kg) and control drug as paracetamol for writhing method while tramadol was used for hot plate method. The young leaves of Solanum melongena was washed with water, air-dried and grinded to a coarse powder. The ethanolic extract was prepared by percolating the dried powder with 95% ethanol and was sent for rotavap to the School of Pharmacy of EAC. Analgesia was measured by acetic acid writhing method and hot plate method. The number of writhings and reaction time were observed and was statistically analyzed using t-test independent followed by ANOVA. In T-test independent all groups even in negative control showed statistical significance. To compare between the group, Anova was done. Again to compare within the groups, post hoc was done. Post hoc showed no analgesic activity among low dose and NSS.

CHAPTER ONE
INTRODUCTION

Herbal medicine has a long tradition of use outside conventional medicine. Herbal medicines are being used for treating and preventing the diseases as there are improvements in analysis and quality control along with the advancement in clinical research. In Philippines, use of such alternative medicine would help the poor people overcome the disease as the herbal medicines are quite cheaper than the conventional medicines.

Solanum melongena is a crop that belongs to the family Solanaceae. It is commonly known as eggplant. It is a much-branched shrub that grows upto 2 m tall and with a long taproot which extends deep into the ground. The stems and leaves are densely covered with star-shaped hairs and sometimes prickles. It has a fleshy berry fruit that is generally smooth and shiny along with many seeds.

Eggplant is cultivated worldwide. Eggplants grow well in warmer parts of the world such as Southeast Asia, South America, and southern Europe.

Eggplant has various medicinal uses therefore it can be valuable addition to the diet. Many studies show that eggplant has nasunin (anthocyanin phytonutrient), a potent antioxidant, that can be found in skin of fruit. Eggplant has being found to lower the LDL because of presence of phenolic compounds in it. Other studies have shown eggplant leaf extract has greater activity against human pathogenic dematophytes or skin fungus.

Eggplants contain flavonoids, alkaloid such as nicotine, saponins and resins. The presence of alkaloids imparted the property of analgesia specially nicotine which is found as an alkaloid in all of S. melongena. The dry residue of S. Melongena leaves shows dose-dependent analgesic effect, attributing to its presence of alkaloids, flavonoids and tannins. In another study, crude samples of S. Melongena roots also showed activities of pain relief with subsequent phytochemical screening test showing compounds flavonoids, alkaloids, tannins and steroids. Hydroalcoholic extract of S. melongena's fruit also showed increase analgesic index when given intraperitoneally, showing prominence in 45 to 60 minutes after administration.

Pain is a more or less localized sensation of discomfort, distress or agony, resulting from the stimulation of specialized nerve endings. Pain consists of both physical and emotional components. Pain can be acute or chronic. Acute pain usually occurs suddenly and has limited duration whereas sometimes pain goes on for weeks, months or even years i.e chronic pain.

Currently, the prevalence of pain worldwide is estimated to be 20% experienced by the adult population, particularly of women. 30-40% from musculoskeletal pain of joint extremities, 30% from neck and back pain, less than 10% from migraine and head pain, and 1-2% of the population experiences pain resulting from cancer. The World Health Organization has estimated that as many as 1 in 10 adult individuals are newly diagnosed with chronic pain each year.

There are various medications for the control of pain. Analgesics are agents that alleviates the pain without causing the loss of consciousness. Mainly the analgesics are classified into NSAIDs and Opioids.

NSAIDs are large, chemically heterogeneous group of drugs that inhibit cyclooxygenase activity, thus results in pain relief. Besides NSAIDs play great role in pain relieving, it has adverse effects too. Use of NSAIDs increases risk of having a range of gastrointestinal (GI) problems. NSAIDs reduce the blood flow to the kidneys. Examples are Ibuprofen, Naproxen, Fenoprofen, Indomethacin, Sulindac, Etodolac, Diclofenac, etc.

Opioids are any compound with opiate like activity but is not derived from opium. Primarily used for pain relief, including anesthesia, opioids are also approved to suppress cough, suppress diarrhea, treat addiction, reverse opioid overdose, and suppress opioid induced constipation. Examples are morphine, methadone, buprenorphine, hydrocodone, and oxycodone.

Paracetamol is a drug that are used to relief pain and fever. It is safe at recommended doses. Paracetamol does not irritate the stomach, as ASPIRIN does, but overdose causes liver and kidney damage and may cause death from liver failure. 15 g or more is potentially serious.

Tramadol is an opioid analgesic used for the treatment of moderate to moderately severe pain following surgical procedures and oral surgery. Mechanism of action include binding to the μ-opioid receptor and also inhibiting the reuptake of serotonin and norepinephrine. It has serious side effects may include seizures, increased risk of serotonin syndrome, decreased alertness, and drug addiction.

➢ *Statement of the Problem*

Will orally administered Solanum melongena leaf ethanolic extract exhibit analgesic activity in male and female Swiss Albino mice?

➢ *Objectives:*

• *General Objectives:*

The study aims to determine the analgesic activity of Solanum melongena leaf ethanolic extract administered orally in Swiss Albino mice using acetic acid induced writhing method and hot plate method.

• *Specific Objectives:*

The study aims to:

✓ Compare the analgesic activity of Solanum melongena leaf ethanolic extract administered orally in Swiss Albino mice using acetic acid induced writhing method and hot plate method with positive control (paracetamol and tramadol respectively)
✓ Determine the dose-response relationship of Solanum melongena leaf ethanolic extract using three different dose levels administered orally in Swiss Albino mice.
✓ Determine the adverse effect upon oral administration of Solanum melongena leaf ethanolic extraction the different dose levels.

➢ *Hypothesis:*

• H_O: *The analgesic activity of Solanum melongena leaf ethanolic extract is comparable to that of paracetamol and tramadol in Swiss Albino mice.*

• H_A: *The analgesic activity of Solanum melongena leaf ethanolic extract is greater than that of paracetamol and tramadol in Swiss Albino mice.*

➢ *Significance of the Study*

This study was done to provide a scientific basis of the analgesic activity of Solanum melongena leaves locally grown in the Philippines. It would be of great benefit to people who use herbal plants for pain relief. Aside from being natural and readily available, it will also help economically those people cultivating Solanum melongena Leaves. People can utilize the plant as a cheaper and safer alternative treatment for alleviating pain.

CHAPTER TWO
LITERATURE REVIEW

In this section, we shall be summarizing previous studies and information of vital importance to our current investigation. This chapter also presents the origin, description, and related literature studies of the eggplant (Solanum melangona)

Historically, medicinal plants have been used since ancient times. Humans commonly used spices because of their high essential oil content that helped to keep food from becoming diseased by bacteria or other microbes. These herbs and spices have special compounds in them to prevent disease. Today we spice food because we think it tastes good, but it is also helps to keep us healthy. These compounds that prevent microbial infection are usually essential oils which we can smell.

Medicinal plants typically have essential oils in their tissues or seeds that prevent bacteria, molds, or other microbes from growing. This quality confers antimicrobial properties. Common herbs like peppermint, basil, oregano, thyme, and rosemary have essential oils that prevent microbial growth. Oregano in particular has been used to help lessen the effects of bacterial infection by making a tea from its leaves.

The eggplant belongs to the Solanaceae, or nightshade family and is known under the botanical name (Solanum melongena L.)(Thompson and Kelly, 1957). It is a semi-tropical plant having two centers of origin. Many botanists believe the larger fruited cultivars originated in the first center which washed Indo-Burma area of plant origins (Boswell, 1949); (Khan, 1979); Nonnecke, 1989.De Candolle (1886) reported in the Origin of Cultivated Plants that the species S. melongena had been known in India from ancient Sanskrit writings. Khan (1979) suggested that these people were probably the first to cultivate it. The ancestral form was very likely a spiny plant with small bitter fruit. Selection over time for improved taste and for relative spinelessness resulted in a more acceptable type (Pierce, 1989; Yamaguchi, 1983).

Eggplant is grown as an annual in the northern hemisphere but in the tropics it is grown as a perennial. A shrubby or bushy plant is characteristic of eggplants but growth is indeterminate that produces new shoots in the leaf axils (Nonnecke, 1989). The result is a plant that gives the appearance of an erect or spreading growth habit. Plants grow to a height of60-120 cm (2-4 ft.). The leaves are large, ovate, and lobed and have spiny hairs on the underside. They grow alternate on the stem. Flowers appear violet-colored, solitary, or in clusters of two or more 2.5 to 3 cm across (Thompson and Kelly, 1957; Nonnecke, 1989). They are similar to tomato but larger. The pendant fruit is a fleshy berry with seeds scattered throughout imbedded in a firm placenta (Pierce, 1989). Most eggplants are self-fertile but occasional crossing and parthenocarpic fruit set does occur.

In fresh weight, eggplants contain about 7% of dry matter (1.4% of proteins, and 4% of carbohydrates); the rest is mostly moisture (Majewska et al., 2009). However, the composition of eggplant varies during storage. Fresh fruit and vegetables are living tissues that unceasingly lose water. Growing crops can replace lost water from the soil, but harvested crops cannot. Consequently, as mentioned above, eggplants will inevitably lose water over time due to transpiration. The rate at which mass losses occur will depend on factors such as harvest, storage, and transportation conditions (Diaz-Pérez, 1998).

Eggplant (Solanum melongena L.) is recognized as one of the most important members of the Solanaceae family which includes economically important species like potato, tomato, tobacco and pepper (Doganlar et al., 2002b; Knapp et al., 2013). Eggplant is grown extensively as cash crop by mostly small-scale farmers in many countries, particularly in Asia. Together with China and India, the Philippines is one of the top 10 eggplant-producing countries in the world based on area of production (FAO Crop Stat, 2012). For the past 10 years, eggplant has been the leading vegetable crop in the Philippines in terms of production volume and area planted (BAS Country Stat, 2012).

S.melangona is herbaceous plant, with coarsely lobed leaves, white to purple flowers, fruit is berry and are grown mainly for food and medicinal purposes. The plant contains flavonoids, tropane, glycoalkaloids, arginine, lanosterol, gramisterol, aspartic acid as important constituents. The plant is reported to have analgesic, antipyretic, antioxidant, anti-inflammatory, antiasthmatic, hypolipidemic, hypotensive, antiplatelet, intraocular pressure reducing, and CNS depressant and anaphylactic reaction inhibitory activities. (Quisumbing, 1969).

At present brinjal eggplant is the third, after potato and tomato, most important crop from Solanaceae family. Greatest eggplant producers are China (17 mln tons per year), Egypt (1 mln tons) and Turkey (0.9 mln ton) (FAOSTAT Data 2006). In Poland, like in many Central European countries, eggplant is still an exotic vegetable but in Asia and the Mediterranean it is an important and valuable nourishment component, the so-called 'the king of vegetables'

S. Melangona was tested for the effect of crude alkaloidal fraction isolated from leaves of Solanum melongena on the central nervous system. It exhibited significant analgesic effect. (Vohra, 1984)

According to Dr. Hautea, eggplant is the most important vegetable crop in the Philippines. She said numerous studies confirmed that eggplants with the Bacillus thuringiensis (Bt) gene are safe for human consumption and the environment.

S. melongena that present symptoms of over maturity, rotting, sunken spots, sunburns, decay, damages due to insects, wounds, cracks, or any other signal of physical injury, should also be rejected in the field or before they are packed to be transported. The fruits are sometimes put in paper bags or wrapped in paper before being packed for shipping (Lawanda and Chavan, 1998).

CHAPTER THREE
MATERIAL AND METHODOLOGY

➢ *Materials and Instrumentation*

- *Basin*
- *Oven*
- *Electric Blender*
- *Large Container Glass Jar*
- *Rotary Evaporator*
- *Electronic Weighing Scale*
- *Mice Cages*
- *Gavages*
- *Test Tubes*
- *Test Tube Racks*
- *Stirring Rods*
- *Graduated Cylinders*
- *Tuberculin Needles*
- *Stopwatch*
- *Heat-Proof Beakers*
- *Electric Stove*
- *Thermometer*
- *Scalpels with Blade*
- *Pins*
- *Gloves*
- *Tissue*
- *95% Ethanol*
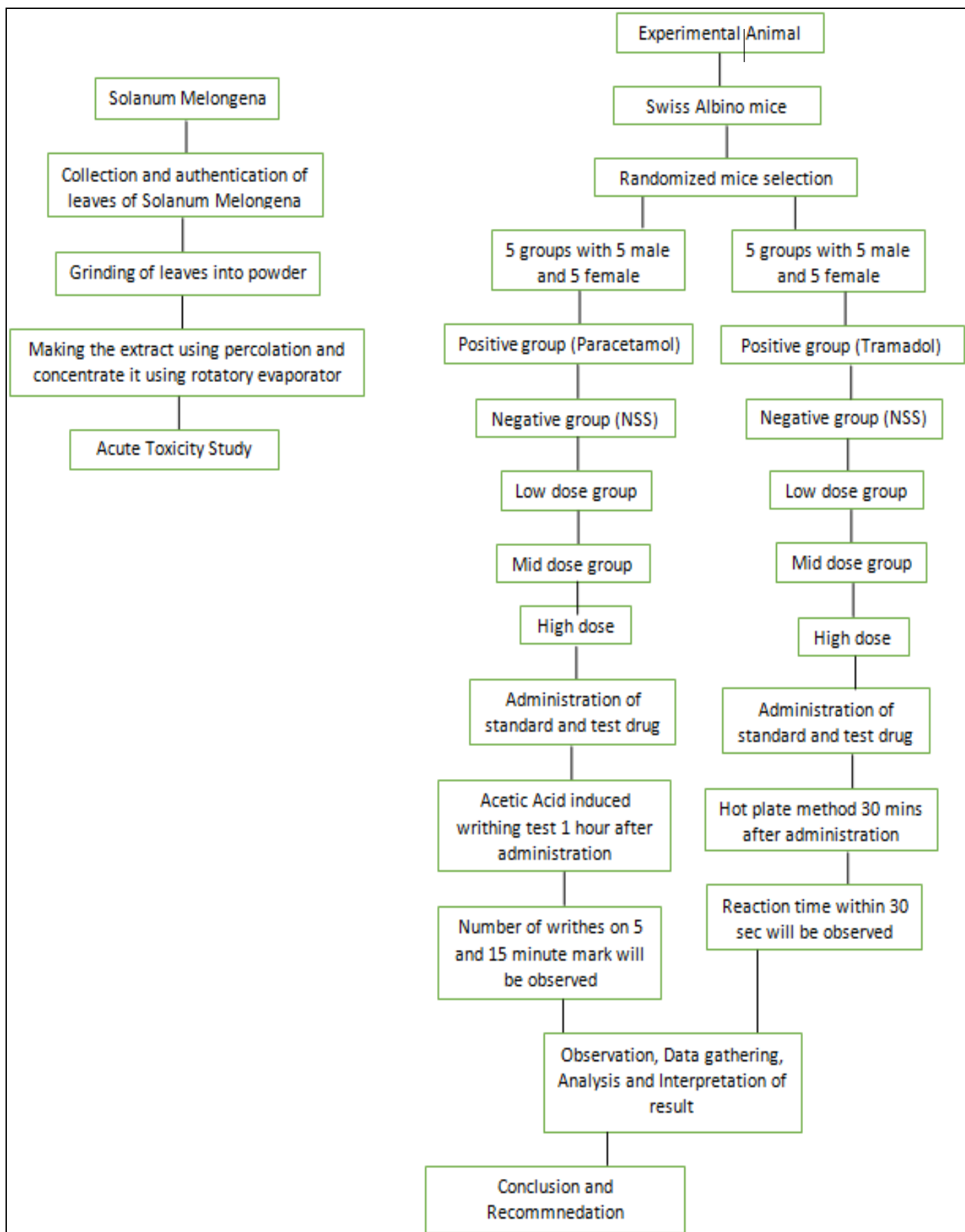- *Distilled Water*

➢ *Research Design*

Fig 1 Research Design

- *Plant Sample*

Twenty kilograms of fresh Solanum melongena leaves were acquired from an organic farm in Paluig, Zambales. It was authenticated by the Botany Department of the National Museum of the Philippines.

- *Experimental Animals*

From sample size calculation we got 10 mice in each group. We used two methods (hot plate and writhing). Each method had 5 groups (positive control, negative control, low dose, middle dose and high dose). So, one hundred Swiss albino mice, 50 males and 50 females, was used for the experiment. The animals will weigh between 15 to 25 grams each. They were sheltered in the animal house provided with proper ventilation and confined within aluminum wire cages. Food and water was also be adequately provided. The animals were acclimatized for five days prior to the experiment.

- *Plant Collection*

Fresh leaves of Solanum melongena spp. was be acquired from an organic farm in Zambales. A plant sample was then be sent to the National Museum for authentication.

- *Plant Extraction*

The leaves collected were washed twice with running water. It was then be air dried for 5 days. When completely dried, the leaves were grinded to fine powder. The powder obtained was subjected to percolation using ethanol as the solvent for 48 hours. Using cheesecloth, the powder was filtered and the volume of the extract was obtained. The extract was then concentrated with the use of a rotary evaporator. After concentration, the volume of the product was obtained once again.

➤ *Acute Toxicity Testing*

- *Confirmatory phase*

Acute oral toxicity of solanum melongena l. was done to determine the NOAEL of the extract. The aim of the test was to determine the range between the dose that causes no adverse effect and dose that is life threatening. A total of 16 swiss albino mice were divided into four groups through random selection: Negative control (NSS), Low dose (3.1gm/kg), Middle dose (12.47gm/kg) and high dose (49.7gm/kg). From the result of confirmatory test, high dose and middle dose had 50% mortality while low dose showed 25% mortality. No Adverse effect (NOAEL) was observed at 0.3ml. The LD50 obtained was 12.29 gm/kg and thus one fourth of obtained LD50 was used as low dose in our experiment proper.

The animals was acclimatized in the laboratory for 5 days and fasted for 12 hours prior to experiment. The extract was administered through oral gavage. Each mouse was placed in metal cage and was observed for 8 hours in first day.

The LD50 of Solanum melongena linn leaves, obtained from a study conducted in EAC Department of Pharmacology 2014, was found to be 12.647gm/kg. We did the confirmatory acute toxicity to confirm this LD50 and it was found to be 12.29gm/kg.

The sample size was determined using the Snedecor and Cochran's formula:

$$n= 1+2C(s/d)^2$$

Wherein, s is the standard deviation, d is the effect size and C is a constant dependent of the selected α and β; standard deviation and effect size were obtained from previous study. Note that α=0.05 and 1- β = 0.8. Thus:

$$n= 1+2(7.85)(0.19/0.25) = 10$$

Thus, sample size of each group is 10, which was composed of randomly assigned 5 males and 5 females Swiss albino mice.

- *Animals*

100 Swiss albino mice, 50 males and 50 females, were used for the experiment and the animals were acclimatized for 5 days. 50 mice for writhing test and 50 for hot plate method. They were kept in the animal house with proper ventilation and food and water provided. One day prior to the experiment, the animals were fasted for 12 hours.

- *The Animals were Randomly Grouped to as follows:*

✓ Group 1: Acetic acid-induced writhing test with NSS
✓ Group 2: Acetic acid-induced writhing test with Paracetamol
✓ Group 3: Acetic acid-induced writhing test with Low dose plant extract
✓ Group 4: Acetic acid-induced writhing test with Middle dose plant extract
✓ Group 5: Acetic acid-induced writhing test with High dose plant extract
✓ Group 6: Hot plate method test with NSS

- ✓ Group 7: Hot plate method test with Tramadol
- ✓ Group 8: Hot plate method test with Low dose plant extract
- ✓ Group 9: Hot plate method test with Middle dose plant extract
- ✓ Group 10: Hot plate method test with High dose plant extract

- *Animal Hygiene*

Animals were kept in well ventilated room in animal house before the experiment. They were given proper animal food and water and hygienic environment was maintained by cleaning the area and keeping each individual in separate cage. Prior to experiment they were fasted.

- *Animal Killing*

The mice that were used in experiment was sacrificed by cervical dislocation procedure.

- *Animal Disposal*

Dead animals were placed in proper container filled with formalin attached with a net and a yellow biohazard bag available at the pharmacology laboratory and was later subjected for proper waste disposal in animal house.

- ➤ *Experiment Proper*

- *Hot Plate Test*

Hot plate test is carried out using hot plate method in order to assess the analgesic activity of the extract of Solanum melongena. Initially, a period of acclimatization of mice in the testing room is provided (30-60 min). The mice were weighed after fasting for 18 hours prior to the experiment and divided into 5 groups consisting of 10 mice for each group. The different substances for each group will be administered via oral gavage.

The temperature of the hot plate, where a mouse will be placed, is regulated at $55º \pm 1ºC$. Hot plate latency will then be observed. Licking of the paws or jumping out of the hot plate was taken as an indicator of the animal's response to heat-induced pain stimulus. If there is no response within 30 seconds, the mouse is removed from the hotplate. Reaction time for each mice will be recorded in seconds. At the end of the experiment, mice is sacrificed.

Table 1 Oral Administration of Substances

GROUP	SUBSTANCE	Dosage
Negative Control	Normal Saline Solution	
Postiive Control	Tramadol	50 mg/ml
Low dose	Solanum melongena leaf ethanolic extract	3.04gm/kg
Middle dose	Solanum melongena leaf ethanolic extract	7.58gm/kg
High dose	Solanum melongena leaf ethanolic extract	19.05mg/kg

- *Acetic Acid – Induced Writhing Test*

Acetic Acid – Induced Writhing Test is done by injection of irritants (acetic acid) into the peritoneal cavity of mice. The mice were weighed after fasting for 18 hours prior to the experiment and divided into 5 groups consisting of 10 mice for each group. The different substances for each group was administered via oral gavage.

An hour after the substance administration, intraperitoneal injection of 1% acetic acid is done to induce pain in each mouse. Number of stretching reaction or writhing movements of the mice will be evaluated for 30 minutes. At the end of the experiment, mice is sacrificed.

Table 2 Oral Administration of Substances

GROUP	SUBSTANCE	Dosage
Negative Control	Normal Saline Solution	
Positive Control	Paracetamol	120 mg/5ml
Low dose	Solanum melongena leaf ethanolic extract	3.04gm/kg
Middle dose	Solanum melongena leaf ethanolic extract	7.58gm/kg
High dose	Solanum melongena leaf ethanolic extract	19.05mg/kg

- ➤ *Statistical Analysis*

Data was expressed as mean values and was evaluated using one way analysis of variance (ANOVA) to determine the difference among the groups. Independent T test was used to determine the difference between each group. Differences between means were considered statistically significant at $p<0.05$ for hot plate method and writhing test.

CHAPTER FOUR
RESULTS

➢ *Hot Plate Test*

Table 3 Analgesic Activity of EESM assessed by Hot Plate Test

	In 30 minutes	SD (30 minutes)	After 1 hour	SD (After 1 hour)
Negative Control	5.71	1.05	5.42	1.07
Positive Control	29.54	0.80	29.89	1.27
Low Dose	9	1.89	9.02	1.46
Middle Dose	18.17	1.27	19	1.06
High Dose	20.63	1.09	22.23	1.43

The above table 3 shows the Analgesic activity of EESM assessed by Hot plate test in 30 mins and after 1 hour and its standard deviation.

➢ *After 30 MINS:* Mean Reaction Time For Different Drug Dose Levels
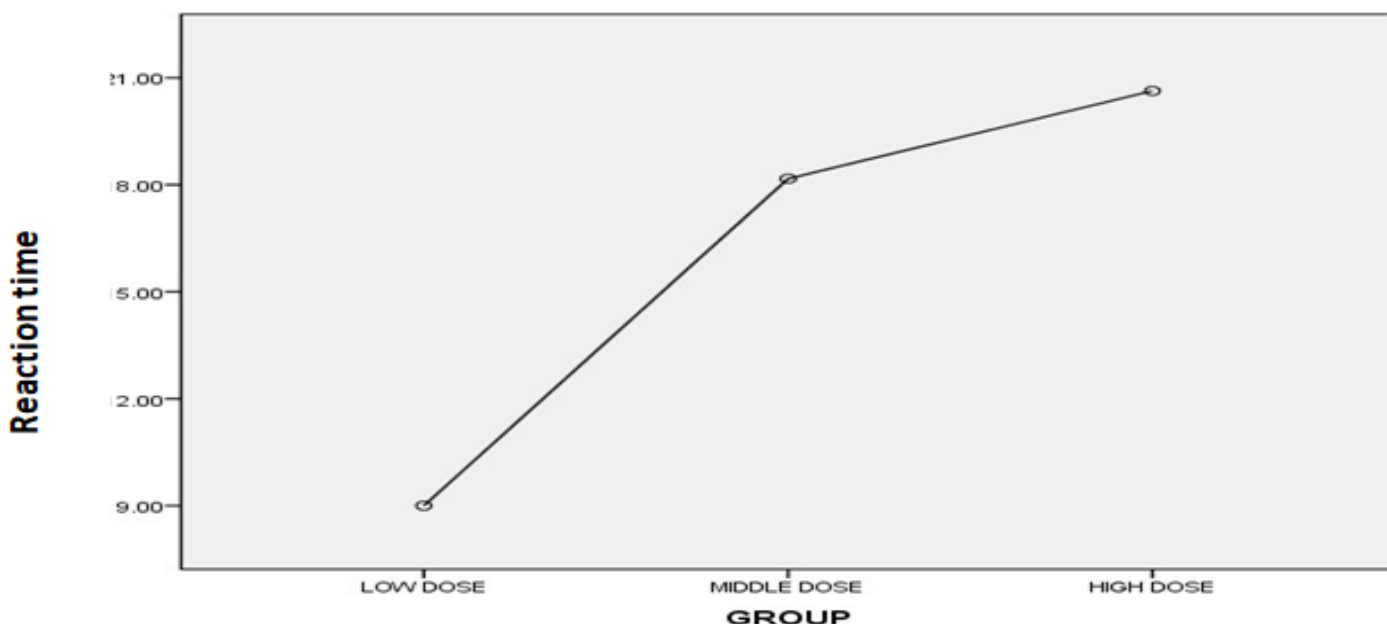
Fig 2 Drug-Dose response curve of SMLEE in hot plate method(30mins)

The above Drug-Dose response curve shows that with the increase in the dose of SMLEE the analgesic activity also increased hot plate method after 30mins.

Table 4 T-Test Independent for Hot Plate Test (30 Minutes)

TREATMENT GROUPS		T computed	T value	p-value
Negative Control	Positive Control	-57.29	1.73	0.00001
	Low Dose	-4.84	1.73	0.00006
	Middle Dose	-25	1.73	0.00001
	high Dose	-31.08	1.73	0.00001
Positive control	Negative control	57.29	1.73	0.00001
	Low dose	31.7	1.73	0.00001
	Middle dose	23.98	1.73	0.00001
	High dose	1.56	1.73	0.001
Low Dose	Negative control	4.83	1.73	0.000067
	Positive Control	-31.67	1.73	0.00001
	Middle Dose	-12.73	1.73	0.00001
	High Dose	-25.58	1.73	0.00001
Middle Dose	Negative Control	24.01	1.73	0.00001
	Positive Control	-23.98	1.73	0.00001
	Low Dose	12.73	1.73	0.0001

High Dose	High Dose	-16.67	1.73	0.0001
	Negative Control	31.08	1.73	0.00001
	Positive Control	20.71	1.73	0.01
	Low Dose	25.44	1.73	0.00001
	Middle Dose	16.66	1.73	0.00001

Table 4 shows that the T computed values for the EESM do not fall within the range of -1.73 to +1.73 and the p-values are less than α. So, our statistical result is significant which means each group shows analgesic activity.

Critical F= dfb, dfw
= (k-1) (N-k), where k= no. of groups & N= total number of sample
= (5-1) (50-5) = 4,45 = 2.58

➢ *Hot Plate (30MINS):*

Table 5 ANOVA for Hot Plate test (30 mins)

DOSE	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3625.010	4	906.252	556.400	.000
Within Groups	73.295	45	1.629		
Total	3698.305	49			

The above ANOVA table 5 is for Hot Plate test (30 mins).The Sig. value in our experiment is 0.00001. This value is less than .05. Because of this, we can conclude that there is a statistically significant difference between the mean numbers of groups. The F computed (556.400) is more than the critical F value (2.58)

Table 6 Post Hoc Turkey HSD for Hot Plate test (30 mins)

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Negative Control	Positive Control	-23.83000*	.57075	.241	-25.4518	-22.2082
	Low Dose	-3.29000*	.57075	.000	-4.9118	-1.6682
	Middle Dose	-12.46000*	.57075	.000	-14.0818	-10.8382
	High Dose	-14.92000*	.57075	.000	-16.5418	-13.2982
Positive Control	Negative Control	23.83000*	.57075	.241	22.2082	25.4518
	Low Dose	20.54000*	.57075	.000	18.9182	22.1618
	Middle Dose	11.37000*	.57075	.000	9.7482	12.9918
	High Dose	8.91000*	.57075	.000	7.2882	10.5318
Low Dose	Negative Control	3.29000*	.57075	.000	1.6682	4.9118
	Positive Control	-20.54000*	.57075	.000	-22.1618	-18.9182
	Middle Dose	-9.17000*	.57075	.000	-10.7918	-7.5482
	High Dose	-11.63000*	.57075	.000	-13.2518	-10.0082
Middle Dose	Negative Control	12.46000*	.57075	.000	10.8382	14.0818
	Positive Control	-11.37000*	.57075	.000	-12.9918	-9.7482
	Low Dose	9.17000*	.57075	.000	7.5482	10.7918
	High Dose	-2.46000*	.57075	.001	-4.0818	-.8382
High Dose	Negative Control	14.92000*	.57075	.000	13.2982	16.5418
	Positive Control	-8.91000*	.57075	.000	-10.5318	-7.2882
	Low Dose	11.63000*	.57075	.000	10.0082	13.2518
	Middle Dose	2.46000*	.57075	.001	.8382	4.0818

*. The mean difference is significant at the 0.05 level.

The above Post Hoc Turkey HSD for Hot Plate test (30 mins) shows the significant value within the five groups that are being compared are significantly different. But in negative control and low dose it showed that the P value is >0.05 and it is not significant in those groups as it doesn't show analgesic activity. We conclude that the differences between condition Means are not likely due to chance and are probably due to the experiment proper.

Table 7 T-Test Independent for Hot Plate test (1 hour)

TREATMENT GROUPS		T computed	T value	p-value
Negative Control	Positive Control	-46.76	1.73	0.00001
	Low Dose	-6.33	1.73	0.00001
	Middle Dose	-28.64	1.73	0.00001
	high Dose	-40.40	1.73	0.00001
Positive control	Negative control	46.00	1.73	0.00001
	Low dose	34.31	1.73	0.00001
	Middle dose	21	1.73	0.00001
	High dose	2.81	1.73	0.05
Low Dose	Negative control	6.3	1.73	0.00001
	Positive Control	-34.31	1.73	0.00001
	Middle Dose	-17.57	1.73	0.00001
	High Dose	-30.61	1.73	0.00001
Middle Dose	Negative Control	28.64	1.73	0.00001
	Positive Control	-20.81	1.73	0.00001
	Low Dose	17.57	1.73	0.00001
	High Dose	-16.30	1.73	0.00001
High Dose	Negative Control	40.40	1.73	0.00001
	Positive Control	-2.81	1.73	0.05
	Low Dose	29.76	1.73	0.00001
	Middle Dose	16.44	1.73	0.00001

Table 7 shows that the T computed values for the EESM do not fall within the range of -1.73 to +1.73 and the p-values are less than α. So, our statistical result is significant which means each group shows analgesic activity.

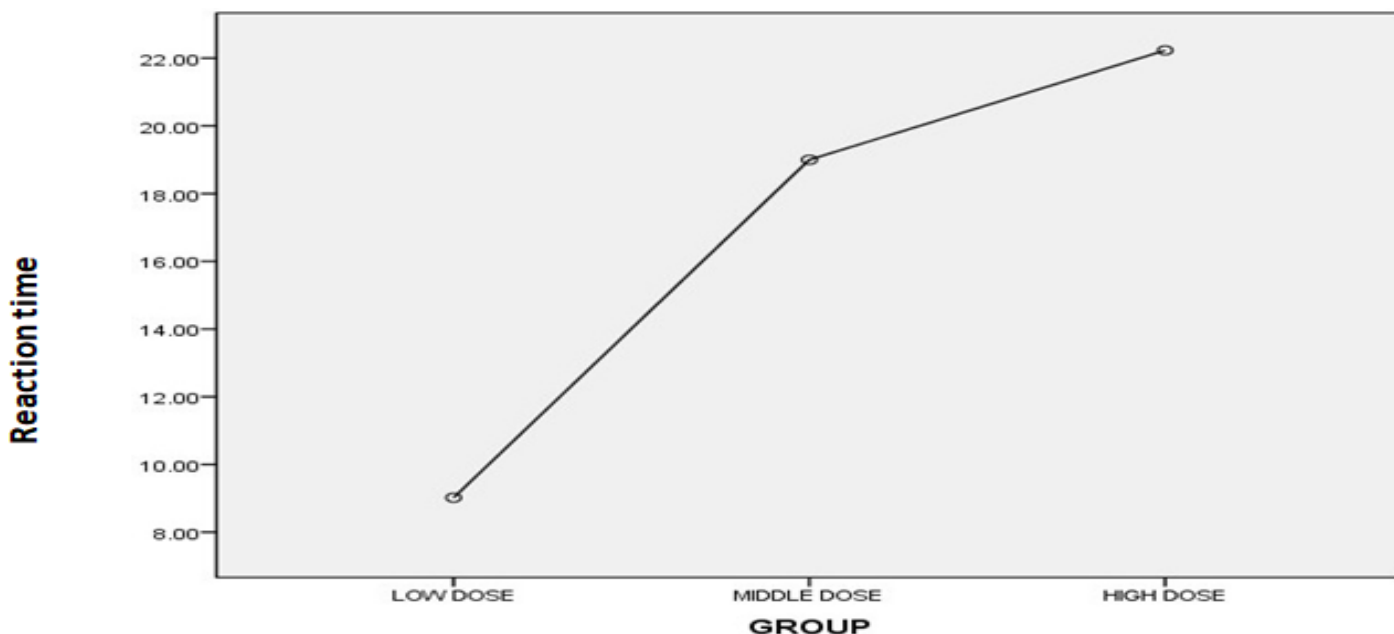➤ *Hot Plate(1 HR):* Mean Reaction Time For Different Drug Dose Levels

Fig 3 Drug-Dose Response Curve of SMLEE in Hot Plate Method(1HR)

The above Drug-Dose response curve shows that with the increase in the dose of SMLEE the analgesic activity also increased hot plate method after 1 hour.

Table 8 ANOVA for Hot Plate test(1hour)

DOSE	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3952.191	4	988.048	747.489	.000
Within Groups	59.482	45	1.322		
Total	4011.673	49			

The above ANOVA table 8 is for Hot Plate test (1hour).The Sig. value in our experiment is 0.00001. This value is less than .05. Because of this, we can conclude that there is a statistically significant difference between the mean numbers of groups. The F computed (747.489) is more than the critical F value (2.58). So we reject our null hypothesis.

Table 9 Post Hoc Turkey HSD for Hot Plate test (1HR)

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Negative Control	Positive Control	-24.47000*	.51416	.000	-25.9310	-23.0090
	Low Dose	-3.60000*	.51416	.241	-5.0610	-2.1390
	Middle Dose	-13.58000*	.51416	.000	-15.0410	-12.1190
	High Dose	-16.81000*	.51416	.000	-18.2710	-15.3490
Positive Control	Negative Control	24.47000*	.51416	.000	23.0090	25.9310
	Low Dose	20.87000*	.51416	.000	19.4090	22.3310
	Middle Dose	10.89000*	.51416	.000	9.4290	12.3510
	High Dose	7.66000*	.51416	.000	6.1990	9.1210
Low Dose	Negative Control	3.60000*	.51416	.241	2.1390	5.0610
	Positive Control	-20.87000*	.51416	.000	-22.3310	-19.4090
	Middle Dose	-9.98000*	.51416	.000	-11.4410	-8.5190
	High Dose	-13.21000*	.51416	.000	-14.6710	-11.7490
Middle Dose	Negative Control	13.58000*	.51416	.000	12.1190	15.0410
	Positive Control	-10.89000*	.51416	.000	-12.3510	-9.4290
	Low Dose	9.98000*	.51416	.000	8.5190	11.4410
	High Dose	-3.23000*	.51416	.000	-4.6910	-1.7690
High Dose	Negative Control	16.81000*	.51416	.000	15.3490	18.2710
	Positive Control	-7.66000*	.51416	.000	-9.1210	-6.1990
	Low Dose	13.21000*	.51416	.000	11.7490	14.6710
	Middle Dose	3.23000*	.51416	.000	1.7690	4.6910

*. The mean difference is significant at the 0.05 level.

The above Post Hoc Turkey HSD for Hot Plate test (after 1hour) shows the significant value within the five groups that are being compared are significantly different. But in negative control and low dose it showed that the P value is >0.05 and it is not significant in those groups as it doesn't show analgesic activity. We conclude that the differences between condition Means are not likely due to chance and are probably due to the experiment proper.

➢ *Writhing Test*

Table 10 Analgesic Activity of EESM Assessed by Acetic Acid-Induced Writhing test

Group	Mean No. of writhings	Std. Deviation
Negative control	30.2	2.11
Positive control	11.7	1.49
Low dose	28.7	1.89
Middle dose	21.2	1.75
High dose	14.3	1.77

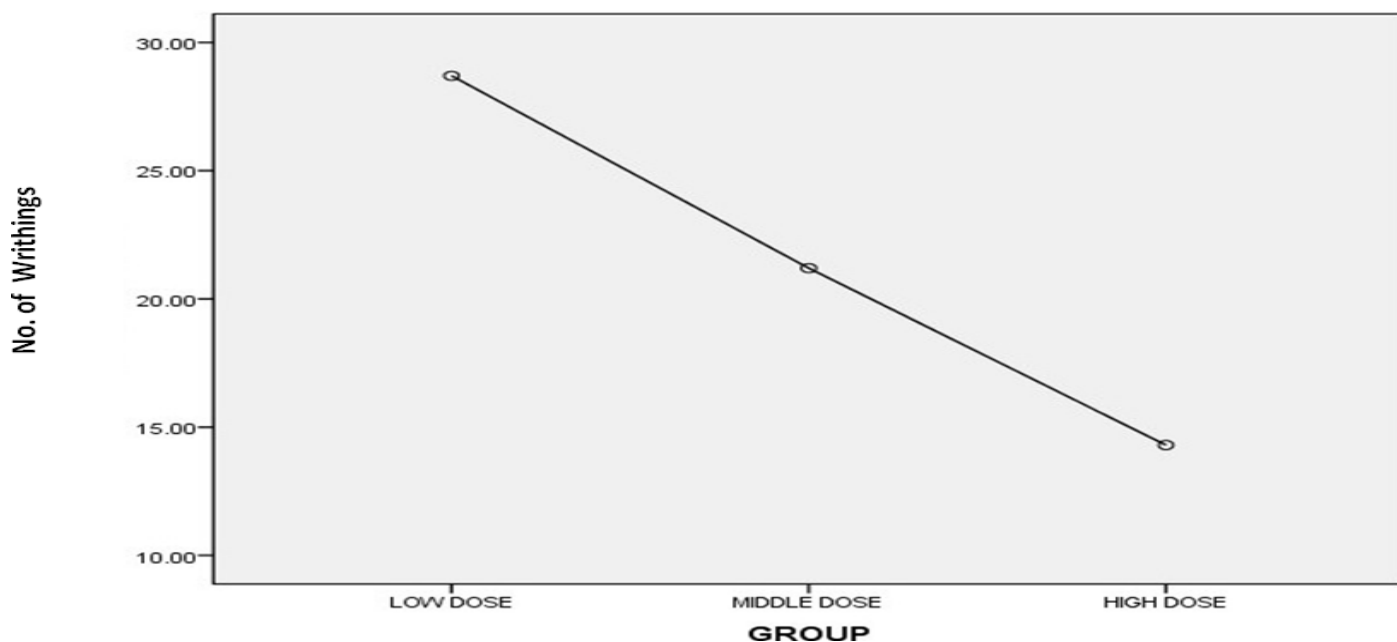➢ *Mean Number of Writhings for Different Drug Dose Levels*

Fig 4 Drug- Dose response curve of SMLEE in writhing method

The above Drug-Dose response curve shows that with the increase in the dose of SMLEE the analgesic activity also increased in writhing method.

Table 11 T-Test Independent for Acetic Acid-Induced Writhing Test

TREATMENT GROUPS		T computed	T value	p-value
Negative Control	Positive Control	22.8	1.73	0.00001
	Low Dose	2.09	1.73	0.02
	Middle Dose	10.37	1.73	0.00001
	high Dose	18.23	1.73	0.00001
Positive control	Negative control	-22.80	1.73	0.00001
	Low dose	-22.0	1.73	0.00001
	Middle dose	13	1.73	0.00001
	High dose	-3.5	1.73	0.002
Low Dose	Negative control	-1.67	1.73	0.05
	Positive Control	22	1.73	0.0001
	Middle Dose	5.73	1.73	0.00001
	High Dose	17.88	1.73	
Middle Dose	Negative Control	-10.42	1.73	0.00001
	Positive Control	13	1.73	0.00001
	Low Dose	-9.31	1.73	0.00001
	High Dose	8.76	1.73	0.00001
High Dose	Negative Control	-18.2	1.73	0.00001
	Positive Control	35	1.73	0.00001
	Low Dose	-17.59	1.73	0.00001
	Middle Dose	-8.77	1.73	0.00001

Table 11 shows that the T computed values for the EESM do not fall within the range of -1.73 to +1.73 and the p-values are less than α. So, our statistical result is significant which means each group shows analgesic activity.

Table 12 ANOVA for Acetic Acid Writhing Method

Values	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2787.320	4	696.830	211.445	.000
Within Groups	148.300	45	3.296		
Total	2935.620	49			

The above ANOVA table 12 is for Acetic Acid Writhing Method. The Sig. value in our experiment is 0.00001. This value is less than .05. Because of this, we can conclude that there is a statistically significant difference between the mean numbers of groups. The F computed (211.445) is more than the critical F value (2.58). So we reject our null hypothesis.

Table 13 Post Hoc Turkey HSD for Acetic Acid Writhing Method

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Negative	Positive	18.70000[*]	.81186	.000	16.3932	21.0068
	Low Dose	1.70000	.81186	.241	-.6068	4.0068
	Middle Dose	9.20000[*]	.81186	.000	6.8932	11.5068
	High Dose	16.10000[*]	.81186	.000	13.7932	18.4068
Positive	Negative	-18.70000[*]	.81186	.000	-21.0068	-16.3932
	Low Dose	-17.00000[*]	.81186	.000	-19.3068	-14.6932
	Middle Dose	-9.50000[*]	.81186	.000	-11.8068	-7.1932
	High Dose	-2.60000[*]	.81186	.020	-4.9068	-.2932
Low Dose	Negative	-1.70000	.81186	.241	-4.0068	.6068
	Positive	17.00000[*]	.81186	.000	14.6932	19.3068
	Middle Dose	7.50000[*]	.81186	.000	5.1932	9.8068
	High Dose	14.40000[*]	.81186	.000	12.0932	16.7068
Middle Dose	Negative	-9.20000[*]	.81186	.000	-11.5068	-6.8932
	Positive	9.50000[*]	.81186	.000	7.1932	11.8068
	Low Dose	-7.50000[*]	.81186	.000	-9.8068	-5.1932
	High Dose	6.90000[*]	.81186	.000	4.5932	9.2068
High Dose	Negative	-16.10000[*]	.81186	.000	-18.4068	-13.7932
	Positive	2.60000[*]	.81186	.020	.2932	4.9068
	Low Dose	-14.40000[*]	.81186	.000	-16.7068	-12.0932
	Middle Dose	-6.90000[*]	.81186	.000	-9.2068	-4.5932

The above Post Hoc Tukey HSD for acetic acid writhing shows the significant value within the five groups that are being compared are significantly different. But in negative control and low dose it showed that the P value is >0.05 and it is not significant in those groups as it doesn't show analgesic activity. We conclude that the differences between condition Means are not likely due to chance and are probably due to the experiment proper.

CHAPTER FIVE
DISCUSSION

Certain noxious stimuli are painful and reflex movements or behaviors resulting from such stimuli are indicative of pain threshold. The stimuli may be thermal, electrical, mechanical or chemical and this informed the adoption of the two analgesic models viz. acetic acid-induced writhing and hot plate methods.

Pheripheral analgesic activity was evaluated by using writhing test in mice according to the method of Koster et al.(1959).The acetic acid induced abdominal contortions or writhing reflex model is a sensitive method for screening analgesic effects of compounds. The writhing reflex seen in this experiment was produced by injection of 1% acetic acid. Intraperitoneal injection of acetic acid produced by injection of acetic acid produces writhing reflex in the animals by activation of chemo-sensitive nociceptors. The percent reduction in the number of abdominal contortions indicates the level of analgesia in the acetic acid writhing reflex model (research journal of pharmaceutical,biologica and chemical science).

The test results in acetic acid induced writhing method showed a decreasing trend in number of writhings as the dose becomes higher but only the high dose showed analgesic activity similar to that of positive control. The test drug at high dose(19.05 mg/kg) body weight showed 14.3 ± 1.49 writhing movement in 20 minutes duration. Mutalik et at. Found the number of writhing with the dried juice of S.melongena Linn. Was 54.66 ± 4.71. Ashish et al.found the number of writhing with the hydro alcoholic root extract of S.melongena was 18 ± 0.34. In the present study standard drug paracetamol produced 11.7 ± 1.49 writhing at the dose of 120mg/5ml.

The result obtained with the test drug in high dose and standard drug were significant when compared with negative control.The analgesic activity shown by middle and low dose of SMLEE were low than that of paracetamol even though they did show more analgesia compared to control group .

On the other hand, the hot plate method is a selective pain test for evaluating centrally acting opioid analgesics like tramadol. A mean of reaction time of tramadol at drug dose of 50mg/ml was 29.54 ± 63 while the mean reaction time of our drug was 20.54 ± 1.09 in high drug dose of 19.05mg/kgOur drug did show increasing analgesic activity with increase in the drug dose. But the analgesic activity of our drug was not comparable to that of tramadol.

SMLEE exhibited both peripheral and central analgesic activity under varying doses. There was dose-dependent response observed in both writhing method and hot plate method. The leaves of S.melongena Linn. Contains flavonoids,alkaloids and tannins. In the earlier study the alkaloid extract of S.melongena was found to produce significant analgesic effect. Various flavonoids both glycosides and aglycones were previously reported as having potent anti-inflammatory and analgesic activity. It is suggested that some flavonoids blocks both cyclooxygenase and lipoxygenase pathway of arachidonate cascade at high concentration,while at low concentration only lipooxygenase pathway is blocked.(asian J. pharm clin Res,Vol 8). There are few reports on the role of tannins in analgesic and anti-inflammatory activity. Previous studies suggested that alkaloids also involve in analgesic action. In the present study the,presence of pain inhibition in the significant doses may be attributable to the fact that SMLEE contains flavonoids and alkaloids which are known to have analgesic activity. SMLEE also exhibited both peripheral and central analgesic activity under varying doses.

No mortality among the mice was observed during the experiment proper.

CHAPTER SIX
CONCLUSION

The ethanolic extract of *Solanum melongena leaves* exhibited analgesic activity. The extract showed comparable activity with paracetamol but not with tramadol. The three dose levels of the ethanolic extract of *Solanum melongena* leaves showed a linear dose-response curve.

➤ *Recommendation*

It is recommended to have a level of expertise in administering the drugs whether orally or intraperitoneally so as to minimize errors and deaths through esophageal perforation, it is also well advised to prepare extra mice as reserves in case of death occurring before the acute toxicity test as well as before the experiment proper begins. Prior to experiment, concentration of all chemicals should be checked and prepared accordingly. A mouse should be kept in one cage with no exception, as well security of each cages should be properly checked to avoid mice escaping.

Table 14 Recommendation

Activity	Week 1	Week 2	Week 3	Week 4
Final Proposal Approval	▮▮			
Sample Collection	▮			
Plant Authentication	▮			
Purchase of Materials	▮			
Plant Washing	▮			
Plant Drying	▮▮▮▮▮▮			
Plant Grinding & Soaking in Ethanol		▮▮		
Rotary Evaporation		▮▮		
Mice Acclimatization		▮▮▮		
Confirmatory Test			▮▮	
Experiment Proper			▮▮▮	
Data Collation & Analysis				▮▮
Research Drafting				▮▮▮▮

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APPENDIX

Table 15 Hot Plate Method

MICE NO.	MICE CODE	WEIGHT (g)	SAMPLE: Drug ADMINISTERED DOSE (ml)	REACTION TIME	
				AFTER 30 MINUTES	AFTER 1 HOUR
NSS					
1	M1	34.3	0.5	5	4.7
2	F1	37.14	0.5	4.2	4.5
3	M2	30.28	0.5	6.3	6.5
4	F2	28.65	0.5	6.1	5.5
5	M3	29.81	0.5	4.8	3.8
6	F3	31.15	0.5	5.3	4.3
7	M4	31.08	0.5	7.9	6.7
8	F4	30.89	0.5	5.7	6.1
9	M5	27.49	0.5	6.5	5.3
10	F5	28.31	0.5	5.3	6.8
TRAMADOL					
1	M6	34.87	0.07	30	30
2	F6	37.14	0.07	30	30
3	M7	32.55	0.07	30	30
4	F7	30.09	0.06	28.5	30
5	M8	33.38	0.07	30	30
6	F8	29.67	0.06	30	30
7	M9	29.44	0.06	29.1	30
8	F9	31.25	0.06	27.8	30
9	M10	42.13	0.06	30	30
10	F10	30.81	0.06	30	28.9
LOW DOSE					
1	M11	21.53	0.32	9.2	8.9
2	F11	30.45	0.46	8.4	8.6
3	M12	31.61	0.48	6.1	7.8
4	F12	29.90	0.45	7	8.5
5	M13	28.77	0.43	8.5	9
6	F13	29.63	0.45	7.9	6.5
7	M14	30.31	0.46	10.5	9.8
8	F14	33.29	0.50	9.9	9.9
9	M15	32.90	0.50	12.8	12.1
10	F15	30.45	0.46	9.7	9.1
MIDDLE DOSE					
1	M16	23	0.87	15.9	17.4
2	F16	30.40	1.14	18.9	18.5
3	M17	25.16	0.85	17	18.7
4	F17	31.27	1.18	16.8	17.9
5	M18	30.83	1.16	19.1	19.5
6	F18	28.63	1.08	18.3	20.6
7	M19	29.15	1.10	18.6	19.3
8	F19	32.72	1.23	17.8	18
9	M20	28.55	1.07	19.6	20.1
10	F20	31.08	1.17	19.7	20
HIGH DOSE					
1	M21	31.89	3.01	20.4	23.4
2	F21	24.53	2.32	19.8	21.3
3	M22	25.11	2.37	21.2	20.2
4	F22	28.67	2.71	21.0	21.5
5	M23	26.91	2.54	20.8	22.8
6	F23	29.60	2.18	22.1	22.0
7	M24	33.35	3.15	20.6	22.4
8	F24	32.28	3.05	19.5	23.3

| 9 | M25 | 27.81 | 2.63 | 18.7 | 20.5 |
| 10 | F25 | 29.50 | 2.75 | 22.2 | 24.9 |

Table 16 Acetic Acid Writhing Method

MICE NO.	MICE CODE	WEIGHT (g)	SAMPLE: Drug ADMINISTERED DOSE (ml)	1% Acetic Acid (ml)	No. of writhing
LOW DOSE					
1	M1	29.12	0.43	0.30	28
2	F1	31.72	0.48	0.32	29
3	M2	20.32	0.31	0.20	26
4	F2	35.37	0.53	0.35	26
5	M3	28.36	0.43	0.28	27
6	F3	36.38	0.55	0.36	30
7	M4	32.82	0.50	0.33	31
8	F4	34.16	0.52	0.34	29
9	M5	27.28	0.41	0.27	30
10	F5	31.04	0.47	0.31	31
MIDDLE DOSE					
1	M6	28.88	1.09	0.29	20
2	F6	26.98	1.01	0.27	21
3	M7	28.35	1.07	0.28	21
4	F7	32.4	1.22	0.32	19
5	M8	37.72	1.42	0.38	23
6	F8	26.09	0.98	0.26	22
7	M9	30.91	1.16	0.31	21
8	F9	29.47	1.11	0.29	25
9	M10	27.63	1.04	0.28	20
10	F10	31.69	1.19	0.32	20
HIGH DOSE					
1	M11	30.94	2.9	0.31	13
2	F11	33.33	3.2	0.33	15
3	M12	30.3	2.9	0.30	15
4	F12	34.92	3.3	0.35	16
5	M13	34.29	3.2	0.34	13
6	F13	28.61	2.7	0.29	14
7	M14	31.05	2.9	0.31	18
8	F14	27.55	2.6	0.28	12
9	M15	29.38	2.8	0.29	13
10	F15	33.86	3.2	0.34	14
PARACETAMOL					
1	M16	31.10	0.39	0.31	9
2	F16	21.11	0.26	0.21	12
3	M17	26.02	0.33	0.26	13
4	F17	33.30	0.42	0.33	11
5	M18	29.81	0.37	0.30	11
6	F18	24.61	0.31	0.25	10
7	M19	28.39	0.35	0.28	14
8	F19	30.08	0.38	0.30	12
9	M20	32.61	0.41	0.33	13
10	F20	27.35	0.34	0.27	12
NSS					
1	M21	25.60	0.5	0.26	32
2	F21	37.80	0.5	0.38	28
3	M22	21.80	0.5	0.22	30
4	F22	31.82	0.5	0.32	31
5	M23	29.28	0.5	0.29	29
6	F23	33.96	0.5	0.34	29
7	M24	30.41	0.5	0.30	35
8	F24	28.61	0.5	0.29	32

| 9 | M25 | 29.77 | 0.5 | 0.30 | 29 |
| 10 | F25 | 30.91 | 0.5 | 0.31 | 29 |

➢ *Preparation of 1% Acetic Acid*

To compute for amount of 99.5% AA to make 10ml of 1% AA:

$$V_1 = \frac{C_2'' V_2''}{C_1} = \frac{(1\ \%\ AA)(10\ ml\ solution)}{99.5\ \%\ AA} = 0.1\ ml\ of\ 99.5\%\ acetic\ acid$$

➢ *Budget of the Study*

Table 17 Budget of the Study

Materials	Quantity/ pieces	Price
Solanum melongena leaves	22.5kg(1kg=200)	4500
0.9% NSS	3	C/o laboratory
Paracetamol	2	c/o Laboratory
Tramadol	2	C/o laboratory
95% Ethanol	4L	c/o Laboratory
Tuberculin Syringe	15 pieces	C/o laboratory
Oral Gavage	5 Pieces	C/o Laboratory
Plant Authentication	-	P80
Total	-	P4580