MEKELE UNIVERSITY

COLLEGE OF VETERINARY SCIENCE

FORMULATION OF USEFULL LOCAL ANESTHESIA FROM *DATURA STRAMONIUM* USING ALBINO MICE

By

Mengstom G/her

CVM pr014/12

A Thesis Submitted to the College of Veterinary Medicine, Mekele University in Partial Fulfillments of the Requirement for the Degree of Master of Veterinary Surgery and Diagnostic Imaging

Adviser

Guesh Negash (DVM, MVSC), Associate Professor

Co adviser

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February, 2024

Mekelle Ethiopia

“DECLARATION`”

I declare that this thesis presents the work carried out by myself and does not incorporate without the acknowledgment of any material previously submitted for a degree or diploma in any university; and to the best of my understanding, it does not contain any materials previously published or written by another person except where due reference is made in the text; all substantive contributions by others to the work presented, including jointly authored publications, are clearly acknowledged.

Name of the candidate Mengstom G/her Signature & Date ____________________

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ADVISOR APPROVAL SHEET

This is to certify that the thesis entitled "Formulation of useful local anesthesia from D*atura stramonium* using albino mice" is submitted in partial fulfillment of the requirements for the degree of Masters of Science in Veterinary Surgery and Diagnostic Imaging has been carried out by Mengstom G/her [CVM/pr014/12] under my supervision. I confirm that the student has fulfilled the requirements and hence can submit the thesis to the Department.

Advisor:

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Signature Date

Co advisor:

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Signature Date

EXAMINERS APPROVAL SHEET

This is to certify that the thesis prepared by Mengstom G/her [CVM/pr014/12], entitled "Formulation of useful local anesthesia from D*atura stramonium* using albino mice" submitted in partial fulfillment for the requirement Masters of Science in Veterinary Surgery and Diagnostic Imaging complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Chair of Department Or Graduate Program Coordinator	____________________	___________________	____________

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TABLE OF CONTENTS **PAGES**

LIST OF FIGURES

LIST OF TABELS

ABBREVIATIONS

ANOVA	Analysis of variance
B/N	Between
CM	Centimeter
CNS	Central nervous system
CONC	Concentration
GM	Gram
LA	Local anesthesia
MG	Milligram
MIN	Minute
ML	Milliliter
MU	Mekelle university
MV	Mill volt
MP	Medicinal plants
RCD	Randomized complete design
SEC	Second
SEM	Standard error of the mean
SPSS	Statistical package for social science
TFL	Tail flick latency
TNS	Transient neurological symptoms

ABSTRUCT

An experimental study was conducted from August to February 2024 to formulate a useful local anesthesia from *D. stramonium* using albino mice. In this study16 adult healthy albino mice were used. After collection the plant the leaves of this plant were subjected for drying under shade of at room temperature. The dried leaves were grounded to coarse powder then, dissolved in 70% ethanol and filtered with bright man filter paper. It was subjected to drying hot oven to evaporate the solvent and the crude extract was again dissolved in sterile distilled water for anesthetic formulation. A total of 16 mice were randomly grouped into four groups to see anesthetic effect. (G-I= injected with 0.9% saline water; G-II= treated with the concentration of 5mg/ml extract of *D. stramonium*; G-III= treated with 7mg/ml of *D. stramonium* G-IV-treated with the standard drug lidocaine2%, each group containing four mice. In this study, ,Group One (Group: I), mice injected with 0.9% saline water served as negative control and mice treated with lidocaine2% served as (positive control) for comparison of the anesthetic activity of D. *stramonium*. Data analysis were done using SPSS version 20, and the results were statistically analyzed using one-way ANOVA and the Post hoc Tuckey's test. Results were presented as mean standard error of the mean, with $P< 0.05$ statistical significance and $p>0.05$ statistical insignificance. Administration of the leaf extract of *D.stramonium*at concentration of 5mg/ml and 7mg/ml exhibited significantly increased ($p<0.01$) tail flick reaction time as compared to the mice group treated with saline water. There were no significant differences in the tail flick reaction between the concentration of 5mg/ml and 7mg/ml of the extract ($p>0.05$).Unlike to this there was significantly increased anesthetic activity ($p<0.01$) of the mice group treated with lidocaine2% compared to the other groups,. The findings of the present study use of *D. stramonium* leaf extract as anesthetic agent in mice is found to be positive, at a concentration of 5mg/ml and 7mg/ml. Therefore, this experiment supported the traditional use of the plant as local anesthetic agent. However, further study is recommended to evaluate the anesthetic effect of *D.stramonium* at various dose formulations and it should also be evaluated with different solvent fractions.

Key words: Anesthetic, D. *stramonium*, Extraction, Formulation, Local anesthesia, mice

CHAPTER ONE

1. INTRODUCTION

1.1 Back Ground of the Study

Pain is a complex phenomenon influenced by various factors, including physical, emotional, and psychological components (Świeboda, *et al*., 2013). It can vary in intensity, location, and characteristics, such as sharp, dull, throbbing, or burning. .Pain can serves as a warning signal that something is wrong or damaged within the body and prompts individuals to take action to protect them and seek appropriate medical attention. Managing pain effectively is an important aspect of healthcare (Bourke, 2014). Treatment approaches may include medications, physical therapy, psychological interventions, lifestyle modifications, and complementary therapies.

Anesthesia is a medical technique used to block pain and induce a loss of sensation during surgical procedures or other medical interventions (White, *et al*., 2007). It helps in the medication of pain by temporarily interrupting the transmission of nerve signals from the site of pain to the brain, thereby preventing the brain from perceiving pain sensations. The term "anesthesia" is derived from the Greek word," aesthesis" meaning without sensation.

There are two basic types of anesthesia; general and local anesthesia. General anesthesia refers to a condition, in which there is a temporary loss of sensation or feelings, particularly the loss of pain sensation. It involves the use of drugs and techniques to induce a controlled state of unconsciousness or numbness, allowing medical procedures to be performed without the patient experiencing discomfort (Bischoff, and Rundshagen, 2011). Local anesthesia refers to local blockade of never conduction whereas general anesthesia involves inducing deep unconsciousness with a depressant drug (Maher, 2012).

Local anesthesia (LA) can be defined as loss of sensation in a discrete region of the body caused by disruption of impulse generation or propagation. LA can be produced by various chemical and physical means. However, in routine clinical practice, local anesthesia is produced by a narrow class of compounds, and recovery is normally spontaneous, and complete.

The medical specialty of anesthesia is essential to various diagnostic or therapeutic procedures, pain management, and surgical interventions (Sekandarzad, 2017). The demand for medicinal plant is increasing in both traditional and modern system of the medicine due to growing recognition of natural product (Sens and Chakraborty, 2017). They are the sources of bioactive compounds that have the potential to develop into new drug structures in addition to crude drugs. (Newman and Cragg., 2012). Because medicinal plants are herbs they have been used since ancient times for relieving pain caused by disease, injury and surgery, some of them contributed to the development of modern anesthesia (Chidiac, *et al*. 2012).

Datura is among such medicinal plant, it belongs to the Solanaceae family, and this plant originates in Americas but is now found around the world including the warmer regions of North, Central and South America, Europe, Asia, and Africa (Bayih, 2014). Datura contains a set of family marker classes such as tropane alkaloids (secondary metabolites) which is an extremely important tropinic derivative or tropane alkaloid and naturally occurs in several species of the Solanaceae family (Grynkiewicz, and Gadzikowska, .2008). Because of the presence of a variety of secondary metabolites including tannins, flvonoides, alkaloids, tropenoids, scopolamine, atropine, glycosides and steroids, D. *stramonium* is a significant plant (Céspede, *et al*., 2021).

It is used as an analgesic and a relaxant for the muscle pain (anti-muscarinic agent) and also is widely used in the treatment of motion sickness, gastrointestinal spasms, and in preoperative medication as an anesthetic (anti-spasmodic agent) (Ullrich, *et al*.,2017). In addition to this it is acting on the parasympathetic nervous system (used as an anti-cholinergic agent) (Ma, *et al.*, 2015).

It is an annual herbaceous plant (30 - 80 cm in height) characterized by branched stems and broad leaves, easily distinguishable by its special appearance. *Datura stramonium* has broad, pointed leaves that are 10 to 15 cm long and 7 to 10 cm wide. Its roots relatively thick and have round, branched stems. There are 5 to 7 identifiably pointed and toothed lobes in them that are irregular in shape (Rahanandeh, 2022). Since early in life, humans use plants for various purposes, such as food and medicine: many people still use various herbs to treat different ailments. Moreover, is recognizing for its importance as a source of drugs in medicine and pharmacology properties (Ayuba, *et al*., 2021).

1.2. Statement of the Problem

Most local anesthesia which employing in minor surgical operations, like hernia, cesarean session, ruminotomy and other superficial tumors; either on its own or in combined with general anesthesia are synthetic anesthetics . These synthetic anesthetics are commonly used but they are costly and not easily available locally as a result of this, an alternative to synthetic anesthetic is plant anesthetic which is readily available less expensive and accessible (Adebayo, *et al.*, 2017).The high cost and issues of scarcity of certain local anesthetics can limit their accessibility and affordability, particularly in resource-constrained settings; consequently, livestock health care at lower expense may perform their miner surgical operations with no anesthetic application. This lead to difficult in surgical procedure due to pain, and can delay wound healing activity following the surgical procedure.

D. stramonium is a plant with a history of traditional medicinal use, which has been reported to possess various therapeutic properties. It is believed to have anesthetic effect based on traditional knowledge however, scientific evidence regarding its anesthetic activity, mechanisms of action, and potential applications in clinical practice is not known. Thus formulation of *D. stramonium* as local anesthesia is more preferred to synthetic ones because it´s availably, less expensive, accessible and environmentally sustainable.

This investigation can help lower healthcare expenses and decrease drug scarcity for pain management options .Ultimately, this research aims to close the gap between conventional wisdom and contemporary scientific understanding providing a viable path for the creation of natural pain killers. The formulation of *D.stramonium* can substitute to the high cost of local anesthesia which are imported from abroad,.

1.3. Research Questions

What is the anesthetic effect of herbal plant *Datura stramonium* in albino mice?

What is its induction period and duration of desensitization effect of *Datura stramonium* extracts?

What are the effects of different doses *Datura stramonium* extracts as compared to standard anesthetic drug lidocaine2%?

1.4. Significance of the Study

Research on the therapeutic benefits of herbal treatments is essential for evaluating their alleged uses, confirming their efficacy**,** and developing plant-based medications for serious ailments. It is estimated that 90% of livestock treatments in underdeveloped nations involve traditional medicine to address the basic medical needs of the animals (Awulachew, 2021).

The findings of this study may provide information on anesthetic effect of *D.stramoniium.* As a result; attention may be paid for additional research on the activity of anesthetic effect to consider as an alternative of local anesthesia. Therefore, this study was conducted particular to fill the information gap on potential anesthetic effect of leaf extracts of *D.stramoniium*in miner surgical procedures. This study may also serve as a benchmark for other researchers, the society may also be aware of the ability of the plant to produce the desired effects. This investigation can also add to the existing body of scientific knowledge by providing insights into the anesthetic effect of *D. stramonium.*

Traditional Anesthetic of Herbal Medicine: *D.stramonium* has a background in traditional medicine systems. This study can provide scientific evidence to support its traditional use as anesthetic agent. It may promote the preservation and recognition of traditional practices and contribute to the integration of herbal medicine into mainstream healthcare.

Pharmaceutical Medicine: The findings of this study can have implications for the medical and pharmaceutical industry. The extract demonstrated has significant anesthetic activity; thus, it may serve as a valuable source for the development of new local anesthesia formulations as a potential compound for further drug development.

Healthcare Professionals: Healthcare professionals can benefit from this study. The results may help them; make informed decisions about incorporating or recommending the extract as a complementary or alternative pain management option in clinical practice.

Animal Welfare: The findings can have implications for animal welfare. Because the extract has demonstrated significant anesthetic activity, it may provide several ways to manage pain after minor surgery, in animals, potentially reducing suffering and improving their overall well-being

1.5. OBJECTIVES

1.5.1. General objective

The general objective of this study is to formulate a useful local anesthesia from D.*stramonium u*sing albino mice.

1.5.2. Specific objectives

To compare the local anesthetic effect of *D.stramonium* extract with control groupand lidocaine 2%.

To evaluate the local anesthetic effect of *D. stramonium*atdifferent dose formulations.

To evaluate the onset of time, recovery time and duration of anesthetic activity of the leaf extract of *D.stramonium*.

CHAPTER TWO

2. LITRATURE RREVIEW

2.1. Definition of Anesthesia

Anesthesia is the clinical field that deals with the reversible induction of pain insensitivity (Tranquilli,, *et.,al*.,2013). It is an essentially practical subject and although becoming increasingly based on science, it still in veterinary practice, anesthesia has to satisfy two requirements (1) the humane handling of animals and (2) technical efficiency. Humanitarian considerations dictate that gentle handling and restraint should always be employed these minimize apprehension and protect the struggling animal from possible injury (Mekonen, *et al.*, 2023).Technical efficiency is not limited to make the treatment easier for the animal to undergo; it also needs to include safeguarding staff members from kicks, bites, and scratches as well as the possibility of unintentional or intentional self-injection of harmful or addictive substances.

General anesthesia is a reversible, controlled pharmacological intoxication of the central nervous system (CNS) in which the patient does not remember or sense unpleasant or painful events. Analgesia may be produced by centrally acting drugs such as morphine given in doses insufficient to produce relief of pain without loss of consciousness or by substances having a local, transient, selective paralytic action on sensory nerves and nerve endings (Edrich, *et al.*, 2007).Moreover, today it is considered that personnel need protection from the possible harmful effects of breathing low con-contractions of inhalation anesthetic agents. The anesthetist aim is to prevent awareness of pain, provide immobility and relaxation of the skeletal muscles (Heravi, 2022). These objectives need to be met without endangering the patient's safety in the moments leading up to anesthesia.

2.2 Local Anesthesia

Local anesthesia is a medical technique that involves the temporary loss of sensation or pain in a specific area of the body (Maland, 2019). It is achieved through the administration of medication called local anesthetics, which block nerve impulses in the targeted region, preventing the transmission of pain signals to the brain. It is commonly used during various medical procedures to numb a specific part of the body, allowing the patient to undergo the procedure without feeling pain. Unlike general anesthesia, which renders the patient unconscious, local anesthesia only affects the immediate area where it is applied or injected (Brown, *et al.*, 2018). In therapeutic settings, a limited family of chemicals produces local anesthetic, and full recovery usually occurs on its own.

2.2.1. Use of Medicinal Plant for Local Anesthesia

Medicinal plants (MPs) are defined as plants that contain compounds can be used as precursors for drug production or for therapeutic purposes (Gurib, 2006). Any plant having compounds that can be used medically or as starting point for the production of pharmaceuticals is considered a medicinal plant (Alamgir, A, 2017). Plants and herbs have always been recognized as highly efficient and reliable medicines in modern and traditional medical systems. *D. stramonium* is among such medicinal herbs. The fundamental anesthetic qualities of medicinal plants are due to secondary metabolites like alkaloids, saponins, tannins, steroids, flavonoids, phenols and glycosides (Shagal, *.et al.*, 2013).Some researchers carried out some works using plant extracts as natural anesthetic, because it is cheaper, safer and more effective at lower concentrations when compared with chemical anesthetics (Agokei and Adebis, 2010).

Datura stramoniun: belongs to family of Solanaceae, and this family comprises about ten species of *Datura*. The primary active agents in *D. stramonium* is atropine, and scopolamine which has been used in traditional medicine and for recreation over centuries (Shi, *et al., 2022l*).The leaves are generally smoked, either in a cigarette or a pipe. During the late 18th century, James Anderson, the English Physician General of the East India Company, learned of the practice and popularized it in Europe (Gorlitsky, .2012 and Pennacchio, *et al*., 2010).The Chinese also used it as a form of anesthesia during surgery (Nellis, 1997). It is (*D stramonium)* a common African plant used among the uniform men especially the military men for psycho-active effects. The compounds, hyoscine, scopolamine as well as atropine, are hallucinogenic substance that courses denseness, alkaloids found in *D. stramonium* was also reported to be narcotic (Adebayo and Olufayo, 2017).

Taxonomic Classification:

Datura stramonium is classified in to

Kingdom: Plantae

Class: Magnoliopsida

Order: Solanales

Family: Solanaceae

Genus*: Datura*

Species: *Datura stramonium* (Gaire and Subed, 2013).

D.stramonium is the most important medicinal plant in traditional medicine due to its many properties (Khan, *et al.*, 2013). It´s important alkaloids are atropine, hyosine and hyoscyamine. The amount of alkaloids in the plant leaves is less than that of in seeds (Jakabova, 2012). In traditional medicine, it was used to treat many diseases, by mixing the leaves with mustard oil in the treatment of dermal disorders (Abbasi, *et al.*, 2010). They are extensively studied for their pharmacological properties, including decongestant of the respiratory system, stimulation of the central nervous system, treatment of skin infections, toothache, and other dental problems, and relief from pain (Sharma, *et al.*, 2021).The leaf extract of datura is used to relieve pain, whereas seed extract are used for coughs and fevers (Ballabh and chaurasia, 2007**)**. The leaf extracts are also applied in the treatment of wounds, hemorrhages, pain, baldness, parasitic infection and muscle spasms (Preissel and Preissel, 2011).

In modern medicine, the plant is used for its medicinal effects as an anti-spasmodic and bronchodilator agent due to the anti-cholinergic properties of atropine and scopolamine, which act by inhibiting the muscarinic receptor on the smooth muscles of the bronchi and sub mucosal gland cells (Peredery and Persinger, 2004). They use the anti-cholinergic properties of Datura plant to neutralize organophosphate poisonings. Experiments showed their efficacy to eliminate acute poisoning in mice. It has also proved effective in alleviating epilepsy in mice (Bania, *et al.*, 2004).

Figure 1 Picture of *D. sramonium from* Mekelle city.

2.2.2. Types of Local Anesthesia

A typical synthetic local anesthetic molecule is composed of a benzene ring (lipid soluble, hydrophobic) and charged amine group (water soluble, hydrophilic) (de Araujo, *et al*., 2019). These are linked by a chemical chain, which can be either an amide [-NH-CO-] or an ester [-O-CO-]. Amides, with longer shelf-life and low potential for allergic phenomena, are more commonly used than esters. Each LA agent has its own onset time, potency and duration of action ending on physicochemical properties of the drugs such as the aromatic ring structure and hydrocarbon chain length, which determine lipid solubility and hence, potency. Protein binding ability also is affected by molecular structure of the drug. Bupivacaine, which is 95% protein-bound, has a longer duration of action than lidocaine (64% protein bound) (Fournier, *et al*., 2010).

The speed of onset of block is determined by the concentration of molecules of local anesthetic agent present in the non-ionized state, which, in turn, depends on the dose dissociation constant (pKa) of the drug and the pH of the tissues (Columb and MacLennan, 2007). The closer the pKa is to physiological pH, the faster the onset of the LA agent. Local anesthetics are typically classified based on their chemical structure and properties. They are commonly categorized into two main groups: ester type and amide-type local anesthesia (Suzuki, *et al*., 2009).

Ester-type local anesthetics: Ester-type local anesthetics are derived from carboxylic acid esters. They include drugs such as procaine, chloro procaine, and cocaine. These local anesthetics are generally metabolized by plasma esters and their duration of action is relatively shorter compared to amide-type local anesthetics (Harcourt, 2022). The earliest inject able local anesthetic, procaine, and enjoyed extensive use during the first half of the past century, primarily as a spinal anesthetic. Its instability and the considerable potential for hypersensitivity reactions resulted in limited use after the introduction of lidocaine. Concerns regarding transient neurologic symptoms (TNS) associated with spinal lidocaine have renewed interest in procaine as a spinal anesthetic. However, limited data suggest that procaine offers only small advantage with respect to TNS, and spinal procaine is associated with a significantly higher incidence of nausea (Hodgson, et al., 2000).

Amide-type local anesthetics; Amide-type local anesthetics are synthesized from carboxylic acid amides. These local anesthetics are metabolized by hepatic enzymes and they generally have a longer duration of action compared to ester-type local anesthetics. Examples of amide-type local anesthetics include lidocaine, bupivacaine, mepivacaine, and ropivacaine. Lidocaine is the most commonly used local anesthetic. It is used for local topical and regional intravenous applications, peripheral nerve block, and spinal and epidural anesthesia. Although recent issues have led to restricted use of lidocaine for spinal anesthesia, this local anesthetic remains popular for all other applications, including epidural anesthesia (Catterall, and Makie, 2006). As compared to other local anesthetics, lidocaine has several advantages in which it has a rapid onset of action, providing quick pain relief, and it also has moderate duration of action, allowing for prolonged pain control (Lirk, *et al*., 2014). Lidocaine is effective for both surface and deeper tissue anesthesia. Additionally it is safe and well- tolerated when used in appropriate dose.

Both ester-type and amide-type local anesthetics work by blocking nerve conduction at the site of administration, and prevent transmission of pain signals (Yu, *et al*., 2017). They achieve this by inhibiting the influx of sodium ions into nerve cells, thereby preventing the generation and propagation of action potentials. It's important to note that the classification and properties of local anesthetics may vary slightly depending on the specific drug and formulation being used. Local anesthesia is a technique of administering an anesthetic agent to numb a specific area of the body, temporarily blocking the generation and transmission of nerve impulses in that area (Maland, 2019). This allows medical procedures to be performed without significant pain or discomfort for the patient.

2.2.3. Methods of Administering Local Anesthesia

Local anesthesia can be administered through various routes depending on the specific procedure and the area to be anesthetized. The most common route of administrations for local anesthetic includes:

- ✓ **Topical anesthesia:** This type of anesthesia is applied to the surface of the skin or mucous membranes. It typically comes in the form of creams, gels, or sprays and is used to numb the skin and or underlying tissues (Ghavimi, *et al.*, 2015). Examples include lidocaine cream or benzocaine spray. The effects of local anesthesia are typically temporary and wear off as the medication is metabolized or eliminated from the body.
- ✓ **Infiltration anesthesia**: Infiltration anesthesia involves injecting a local anesthetic directly into the tissues near the surgical site. This technique is commonly used for minor procedures such as suturing a wound or removing a small skin lesion.
- ✓ **Nerve block anesthesia**: Nerve block anesthesia involves injecting a local anesthetic near a specific nerve or group of nerves to numb a larger area of the body (Catterall. and Mackie, 2006). It is commonly used for procedures such as joint replacements or surgeries on the limbs. Examples include epidural anesthesia for childbirth or brachial plexus block for upper limb surgery.
- ✓ **Epidural anesthesia**: is a type of regional anesthesia commonly used during child birth and certain surgical procedure. It involves the administration of local anesthetic medication into the epidural space, which is the space surrounding the spinal cord in the spine. This method used for pain relief during labor and

delivery or for surgeries involving the lower abdomen, pelvis, or lower extremities.

- ✓ **Spinal anesthesia** (subarachnoid block): The anesthetic is injected into the cerebrospinal fluid in the subarachnoid space, which surrounds the spinal cord (Stoodley, *et al*., 1996). This method is commonly used for surgeries involving the lower abdomen, pelvis, or lower extremities.
- ✓ **Field block anesthesia**: Field block anesthesia is similar to nerve block anesthesia but involves injecting the local anesthetic around the perimeter of an entire surgical field or region (Suzuki, *et al*., 2019). This technique is often used for procedures such as skin grafts or liposuction.
- ✓ **Intravenous regional anesthesia** (Bier block): It is a method of providing local anesthesia to a specific limb or extremity. This technique involves using a tourniquet to isolate blood flow to a limb and then injecting a local anesthetic into a vein. The anesthetic remains localized to the limb, providing pain relief during procedures such as hand or forearm surgeries. The choice of technique depends on the specific procedure, the desired level and duration of anesthesia, the patient's medical condition, and the preferences of the anesthesiologist or surgeon. Local anesthesia is generally considered safe and effective when administered by trained healthcare professionals (Chou, 2016). It's obvious to understand that while LA reduces discomfort during a procedure, it does not provide sedation or alter ones state of consciousness. In some cases, additional sedation may be provided alongside LA to help keep the patient calm and comfortable during longer or more invasive procedures.

2.2.4. Mechanism of Action of Local Anesthesia

Local anesthetics block the transmission of the action potential by inhibition of voltage-gated sodium ion channels. Under normal or resting circumstances, the neural membrane is characterized by a negative potential of roughly –90 mV (the potential inside the nerve fiber is negative relative to the extracellular fluid) (Matthews, 2002). This negative potential is created by active outward transport of sodium and inward transport of potassium ions, combined with a membrane that is relatively permeable to potassium and relatively impermeable to sodium ions (Ussing, 1949). With excitation of the nerve, there is an increase in the membrane permeability to sodium ions, causing a decrease in the trans-membrane potential. If a critical potential is reached (i.e., threshold potential), there is a rapid and self-sustaining influx of sodium ions resulting in depolarization, after which the resting membrane potential is reestablished.

From an electro-physiologists, local anesthetics block conduction of neural-transmission by decreasing the rate of depolarization in response to excitation, preventing achievement of the threshold potential (Strichartz, *et al.*, 2009). They do not alter the resting trans-membrane potential, and they have little effect on the threshold potential. In order to cause loss pain in a particular area of the body, LA temporarily blocks nerve signals in that area (Maland, 2019). There are various mechanisms of action for local anesthetics, but the most common one involves blocking the generation and conduction of nerve impulses. Local anesthetics are usually delivered topically or by injection, and they function by attaching to particular receptors on the nerve fibers in the affected area. These receptors are called sodium channels, which are responsible for the propagation of nerve impulses (Catterall and Mackie, 2006).

When a local anesthetic binds to the sodium channels, it blocks the influx of sodium ions into the nerve fibers. This prevents the nerve from depolarizing and generating an action potential, which is necessary for the transmission of nerve signals. Local anesthetic prevents the brain from receiving pain signals normally by inhibiting the action potential, resulting in temporary numbness and loss of sensation in the area (Maland, 2019). The binding of local anesthetics to sodium channels is usually reversible; allowing the nerve function to return once the anesthetic is metabolized or diffuses away from the site of action. The duration of action of a local anesthetic depends on various factors such as the specific drug used, the concentration of the drug, and the vascularity of the area being anesthetized (Lirk, et *al.*, 2014).

2.2.5. The Ideal Properties of Local Anesthesia include

Ideal property of local anesthesia refers to the characteristics that make local anesthetic agent effective and safe for use in medical procedure. These properties are:

- **Selective action**: LA should primarily affect the nerves in the target area, while minimizing the impact on the surrounding tissue. This ensures only the desired area is numbed, allowing for precise and effective pain control. It is also produce reversible loss of sensation and motor function in specific area of the body without affecting consciousness or causing systemic side effects (Ferneini, *et al*., 2021). They should block nerve conduction in sensory fibers more readily than in motor fibers.
- **Potency**- LAs should be potent enough to produce the desired effect at a reasonable dosage and concentration. Higher potency allows for lower doses and reduces the risk of systemic toxicity (Wadlund, 2017). This allows effective pain relief without the need for excessive amount of the drug.
- **Rapid onset**: Local anesthetics should take effect quickly to minimize patient discomfort and allow for efficient and timely procedures (Golembiewski, 2013). This for a smoother and more conferrable experience for the patient.
- **Prolonged duration of action**: Local anesthetics should have a sufficient duration of action to cover the expected duration of the procedure and provide postoperative pain relief (Chou, *et al.*, 2016) Prolonged duration of action reduces the need for additional doses or supplementary anesthesia.
- **Reversible effects**: the effect of local anesthetics should be temporary and reversible once the procedure is complete, the anesthesia should wear off relatively quickly allowing normal sensation and function to return to the area.

  Minimal toxicity: Local anesthetics should have a high margin of safety to minimize the risk of toxicity or adverse reactions. They should have minimal systemic effects when used appropriately, especially when administered by the recommended routes (K, 2018)**.**
- **Non-allergenic**: Ideally, local anesthetics should have low allergenic potential to minimize the risk of allergic reactions in patients. However, some individuals may still be sensitive or allergic to certain local anesthetics.
- **Stability:** Local anesthetics should be stable under normal storage conditions to ensure their effectiveness and potency throughout their shelf life.

- **Adequate tissue penetration**: Local anesthetics should be able to penetrate and distribute within the target tissues effectively to achieve the desired anesthetic effect. It's important to remember that no single local anesthetic can perfectly fulfill all these properties perfectly.. The choice of local anesthetic depends on various factors, including the type and duration of the procedure, patient characteristics, and the preferences of the healthcare provider (Hwang, *et al*., 2014). Different local anesthetics have different properties and are selected accordingly to meet the specific requirements of each situation.

2.2.6. Adverse Effect of Local Anesthesia

Although it is rare, important adverse effects of local anesthetics may occur from systemic absorption, local tissue toxicity, allergic reactions, and drug-specific effects. Systemic toxicity of local anesthetics results from excessive plasma concentrations of these drugs, most often from accidental intravascular injection during performance of peripheral nerve blocks (Gitman, *et al*., 2019). Less often, excessive plasma concentrations result from absorption of local anesthetics from tissue injection sites.

The magnitude of local anesthetic of systemic absorption depends on the dose injected, the specific site of injection, and the inclusion of a vasoconstrictor in the local anesthetic solution. Systemic absorption of local anesthetic is greatest after injection for intercostals nerve blocks and caudal anesthesia, intermediate after epidural anesthesia, and least after brachial plexus blocks (Rosenberg, *et al.*, 2004). The risk of accidental intravascular injection and consequent acute toxicity is ever-present with most neural blockade techniques. The strength, dosage, and rate of administration of local anesthetics are strongly correlated with the degree of toxicity to the cardiovascular and central nervous system (CVS and CNS) respectively (Mather, 2010).

Clinically significant of LA toxicity results CNS stimulation, ranging from tremors to convulsions and perhaps cardiac dysrythmias, can be described in terms of a chaos derived state change in which the local anesthetic appears to act as an initiator. Both CNS and CVS effects are rather poorly correlated with arterial drug concentrations but better correlated with concentrations in the respective regional venous drainage (Mather, *et al*., 2005). Establishment of maximal acceptable local anesthetic doses for performance of regional anesthesia is an attempt to limit plasma concentrations that can result from systemic absorption of these drugs.

However, standard dosage recommendations are not evidence-based, are inconsistent, and they fail to take into account the specific injection site and patient-related factors (Rosenberg, *et al.*, 2004). Nonetheless, dosage recommendations represent guidelines for providing a starting point from which adjustments based on clinical circumstances and evolving evidence can be made. Local anesthetic toxicity generally occurs as a result of therapeutic error. Situations leading to toxicity include inadvertent venous or arterial injection as well as too high dose of ingested or topically administered local anesthetic-containing preparations (Paraben, 2010).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Materials

Electrical balance, maceration jar, orbital shaker, measuring cylinder, sacker, bright sign filter paper, drying oven, heater/hot plate, 1ml measuring syringe and needle, were used during the study.

3.2 Chemicals

Lidocaine2%, Ethanol70% (ethyl alcohol) saline water, and distilled water reagent grade ref-5601 LTD- 562306/1L) were used in this study.

3.3 Study Area and Study period

The investigation was conducted in Pharmacognosy and Pharmacology departments' experimental unit and laboratories at Mekelle University (MU) Colleges of Health Sciences. This experimental study was conducted from August 2023 to February 2024.

3.4. Experimental Animals

Swiss albino mice were obtained from the animal house at Mekelle University, Pharmacognosy and Pharmacology Department. Prior to the commencement of the study, the mice were allowed one week to become accustomed to the surroundings of experimental unit. They were kept in individual cage where they can get 12-hour light and dark cycles and they were fed with pellets and water. The mice were placed into four groups for the treatment of anesthetic activity (6-8 weeks old, 28-32g weight). For this treatment mice in group, I was given saline water (as a negative control group), whereas mice in group II received 5mg/ml D. *stramonium* extract. Mice in Group III and IV, 7mg/ml extract of D. *stramonium*, and lidocain 2% (as a standard drug) were given respectively.

3.5 Study Design

An experimental study design with randomized complete design (RCD) was used in which treatment groups were independent variables while the group mice were dependent variable.

3.6 Plant material collection and preparation

The leaves of *D. stramonium* were gathered from Mekelle City in October 2023. The plant materials were then packed in plastic sheets and delivered to department of Pharmacognosy and Pharmacology laboratory at Mekelle University Health Science College. The collected plant materials were washed to remove any debris or dead matter and dried under shade in a clean environment. The dried leaves were grounded to coarse powder using electric grinder then prepared for extraction.

3.7 Plant extraction

The maceration extraction method as outlined by (Muslisa., 2015), was utilized to extract the experimental plant material. A 443gm of the coarse powdered leaves of *D. stramonium* were soaked in 1772 ml of 70% ethanol in a maceration jar for 72hours at room temperature with frequent stirring for 30 min (three days) until the soluble material was dissolved. Bright sign filter paper was used to filter the mixture. The filtered was dried in a drying oven at 40°C to evaporate the solvent. 40g of leaf extract of *D. stramonium* was obtained and used as anesthetic activity. The leaf crude extract was ground to obtain powder form. This powder form of extracted was mixed and dissolved in sterile distil water. Then specified quantity of distilled water was added as required amount to make anesthetic formulation at a concentration dose of 5mg/ml and 7mg/ml.

3.8 Evaluation of Anesthetic Effect of *D. stramonium* using tail flick latency test

The anesthetic activity of *D. stramonium* was evaluated according to the method described by (Sasidharan, *et al*., 2023); part of mice tail exposed to a radiant heat of 55°C, the normal or untreated mice tail reaction time lies between 1to 5.5 seconds. Whereas tail withdrawal time more than or 6 seconds indicates positive to anesthetic agent. The in vivo anesthetic effect of *D. stramonium* was assessed using radiant heat TFL test. Tail flick is the time lapse between the onset of stimulus application and the animals moving their tails away from the heat source (Li, *et al.*, 2013). Tail flick represents the mice's sensitivity towards the temperature. In order to measure the local anesthetic effects on normal skin, treatments were administered subcutaneously to the mouse tail by application of drug solutions at the base of distal portion of the tail. Cut of time should be at 12sec in order to decrease the skin damage of mice tail (Eide, *et al*., 1988).

Animals were grouped and treated in the indicated situation (animals were grouped in four groups, four animals in each group): Group I – Control, normal saline (0.9%) subcutaneous route; Group II – D. *stramonium*test, at concentration5mg/ml; Group III- *D. stramonium*test, 7mg/ml subcutaneous route; Group IV standard drug lidocaine2% subcutaneous route. Tail flick were measured immediately following application and at five-minute intervals until the recovery time. *D.stramonium* 5mg/ml, *D.stramonium* 7mg/ml lidocaine (2%), and normal saline (0.9%) were injected subcutaneously at the base tail of the experimental mice, and anesthetic effect was determined at fixed time intervals (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80 and 90 min). If the mice flick their tail at 6 or more seconds in response to heat stimulation at 55°C threshold, this indicates anesthetic agent; whereas if the mice flick their tail before 6 seconds in response to heat stimulation, it indicates no anesthesia (no anesthetic effect).

3.9 Statistical Analysis

Data entry, coding, and analysis were done using SPSS version 20 (Statistical Package for the Social Sciences). The results were statistically analyzed using one-way analysis of variance (ANOVA) and the Post hoc Tuckey's test. Results were presented as mean standard error of the mean (SEM), with $P < 0.05$ denoting statistical significance whereas $p > 0.05$ denoted that statistical insignificance.

3.10. Ethical Approval

Ethical clearance was obtained from the Animal Ethics and Experimentation Committee, Mekelle University, College of Veterinary Sciences; and the guidelines on care and use of animals for scientific purposes were considered during the experimentation.

CHAPTER FOUR

4. RESULT

Anesthetic effect of *D. stramonium* was investigated in Swiss albino mice by TFL test. In this study, 70% ethanolic extracts of *D. stramonium* administered subcutaneously had significant effect on tail flick latency on the experimental treatment group of mice. Tail flick latency of each treatment groups during this study is shown in (Table 2).

There was a significance increase (P <0.05) in reaction time of tail withdrawal from radiant heat stimulation in the mice group treated with both concentrations (5mg/ml and 7 mg/ ml) of this plant extract as compared to mice group treated with saline water. Although, the rate of reaction time was increased using 7mg/ml dose of *D. stramonium* crude extract, there was no significance difference (p>0.05) between the 7mg/ml and 5mg/ml of extracts in terms of anesthetic activity. Whereas, in mice group treated with lidocaine2% (the standard drug) had significant increase (p< 0.05) in heat tail flick reaction time in comparison to the mice group treated with test extract (5mg/ml and 7mg/ml of D.*stramonium*).Starting from 10min onwards, the group that received lidocaine2% showed a significant difference (P <0.05) in tail flick reaction as compared to the control group and extract test groups. The highest reaction time of D. *stramonium* on test group was 9 sec at 25 min, while it was 3 sec 11 sec for control group and the standard drug lidocaine2% respectively

Table 1 Induction time and recovery time of mice anesthetized with ethanolic extract of D.stramonium.

Treatment group	**Treatment (Dose.)**	**Induction time (min)**	**Recovery time (min)**
1.G-I	Saline water o.9%	In effective	-
2.G-II	D. stramonium5mg/ml	25±4.08	40±2.87
3.GIII	D. stramonium7mg/ml	20±4.08	45±4.08
4.G-IV	Lidocaine2%	10±4.08	90±2.87

The result showed no significant relation between dose (concentration) and induction time (p>0.05) increase in concentration led to decrease in duration of expose to anesthetic (induction time). There is also insignificant relationship between concentration and recovery time, (p>0.05) an increase in anesthetic concentration increase in duration of the mice coming back to active (table 1).*D. stramonium* solution showed anesthetic effect within the first 20minates, with a peak effect seen within 25 minute after the application. In this work, the tail-flick reaction time of mice treated with D. *stramonium (*test group), normal saline water (negative control) and lidocaine2% (standard drug) had recorded different reaction time (figure 2). Mice treated with normal saline (negative control) did not show any significant difference in the reaction time on tail-flick throughout the 90 min observation. In the standard treatment group the anesthetic activity increased rapidly and then progressively recovered to its baseline level, as in the control group. In contrast, the anesthetic activity of test extract group started anesthetic state gradually and recovered sharply with short period of time. Although, the duration of anesthesia in mice group which were administrated subcutaneously with the extract of D. *stramonium* was not comparable to that of the mice group treated with commercial 2% lidocaine, it had visible and clear anesthetic activity (Table 2).

The results showed that with increasing drug concentration, the duration of onset of anesthesia had a decreasing trend. Also, the onset of anesthetic activity was shorter and the return time was increasing. In treatments with a concentration of 5 and 7 mg /ml of extract of D. *stramonium* induced anesthesia in albino mice at 25 and 20 minutes respectively after subcutaneous administration of this test extract. Whereas in lidocain2% treated group anesthetic activity started at 10 minute after application. In mice exposed to 5mg/ml concentration of D.*stramonium* average induction time was 25 min and average recovery time was 40 min after subcutaneous administration. Mice treated with 7mg/ml of the leaf extract began to desensitization of their tail at 20 min and recovered 45 min after application of this crude extract. In injection lidocane2%, mice reached anesthesia within 10 min and they were recovered at 90min.

TFL value represents the mean ±SEM statistical differences were determined by one way ANOVA followed by Tukey´s post hoc test. Compared to saline water *p<0.05, **p<0.01, ***p<0.01 as compared to saline water n=4

Table 2 Showed tail flick reaction (sec.) of four treatment group at five minute interval.

Time in minute	**Treatment group**			
	G-I	**G-II**	**G-III**	**G-IV**
5 min	2.5±2.87	2.5±2.87	2.5±2.87	5±4.08
%	-	-	-	-
10 min	2.5±2.87	2.5±2.87	2.5±2.87	8±4.08**
%	-	-	-	66%
15 min	2.5±2.87	2.5±2.87	4.5±2.87	9.25±4.79
%	-	-	-	77%
20 min	2.5±2.87	3±4.08	7±4.08*	10.25±2.87***
%	-	-	58%	85%
25 min	2.5±2.87	6.25±4.08*	9±4.08**	9.5±2.87**
%	-	52%	75%	79%
30 min	2.5±2.87	8±4.08**	8.5±4.08**	9±4.08**
%	-	66%	70%	75%
35 min	2.5±2.87	7±4.08*	8±4.08*	8.75±0.25
%	-	58%	66%	73%
40 min	2.5±2.87	4±2.87*	7±4.08	8±4.08**
%	-	-	58%	66%
45 min	2.5±2.87	2.75±2.87	3.5±4.08	7.5±2.87*
%	-	-	-	62.5%
50 min	2.5±2.87	2.75±2.87	2.5±2.87	7±4.08*
%	-	-	-	58%
60 min	2.5±2.87	2.75±2.87	2.5±2.87	7±4.08*
%	-	-	-	58%
70 min	2.5±2.87	2.75±2.87	2.5±2.87	6.5±2.87*
%	-	-	-	54%
90 min	2.5±2.87	2.75±2.87	2.5±2.87	4.5±2.87
%	-	-	-	

Figure 2 Tail flick latencies of mice after treatment with D. *stramonium* at concentration of /ml and 7mg/ml at different time intervals. Here there is a comparison with Control: Normal saline, and Standard- lidocaine.

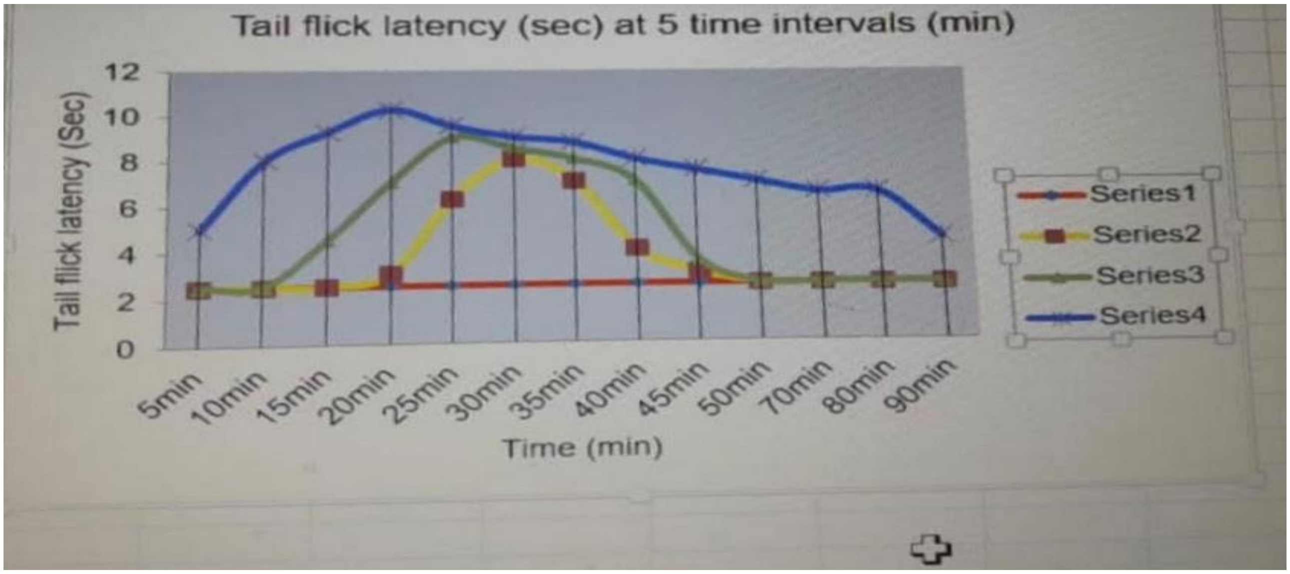

Table 3 Reactions of treatment group at temperature of 55°C; following the subcutaneous administration

Group	TFL in sec. at 5min interval													
	5	10	15	20	25	30	35	40	45	50	60	70	80	90
1. G-I	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2. G-II	+	+	+	+	-	-	-	+	+	+	+	+	+	+
3. G-III	+	+	+	-	-	-	-	-	+	+	+	+	+	+
G-IV	+	-	-	-	-	-	-	-	-	-	-	-	-	+

CHAPTER FIVE

5. DISCUSSION

Local anesthetics are clinically used to control pain during minor surgeries or to treat other acute and chronic pain. It is achieved through the administration of medication local anesthetics, which block nerve impulses in the targeted region, preventing the transmission of pain signals to the brain (Strichartz, *et al*., 2009). It is commonly used during various medical procedures to numb a specific part of the body. Many animal healthcare professionals use local anesthetics to treat painful conditions. In order to manage pain during surgical procedure or relieve pain after surgery, lidocaine, bupivacaine, etidocaine, and other local anesthetics are commonly used in clinical practice by modern medicine.

Various pharmacological components have been identified from natural plant sources, many of which were utilized in traditional medicine. Plant derived medicines have been used in healthcare for thousands of years in medical systems, and as a result, traditional medicine is still widely practiced today. They work by exhibiting, antioxidant anti-asthmatic and anti-cholinergic properties (Khosroukhavar, *et al*., 2010). The present herbal plant anesthetic agent was formulated from *D. stramonium*. It was clear from the literature study that the medicinal plant used in the formulation had previously been reported to have anesthetic effect. Because of the presence of a variety of secondary metabolites including tannins, flvonoides, alkaloids, terpenoids, scopolamine, atropine, glycosides and steroids, *D. stramonium* is a significant plant (Céspedes, *et al*., 2021).

The primary anesthetic effect of *D. stramonium* is due to the presence of scopolamine. Scopolamine is a potent ant-cholinergic agent, which means it blocks the action of the neurotransmitter acetylcholine in the central and peripheral nervous systems (Drevets, .*et al*., 2013). By inhibiting the activity of acetylcholine, scopolamine produces a variety of effects, including sedation, amnesia, and analgesia (Sayhan, *et al*. 2017).In many situations, the effective treatment of pain has been achieved by the use of local anesthetics derived from plant alkaloid.

The most well-known way that local anesthetics work is by blocking motor and sensory processes by interacting with voltage-gated Na+ channels. (Tsuchiya, 2017). To evaluate whether any herbal anesthetics formulation works well in experimental animals, it is important to conduct in vivo test.

Different procedures are used to study the effect of local anesthetic formulations, which vary in terms of their durations and clinical efficacy (Becker, and Reed, 2012).The effectiveness of local anesthetics must be assessed by measuring the level of pain relief following drug application using radiant heat stimulation. This technique is frequently used to measure pain following the application of any type of anesthetic agent. Tail flick perspective approach is the most widely used method for evaluating the degree of acute or chronic pain (Ouchi, *et a*l, .2013). It employs thermal heat to assess the pain threshold and the effect of anesthesia. Thermal heat is a type of constant infringement stimulation that does not harm the skin when a proper cut-off time is used (Yang, .2019).

In the current investigation, the extract treatment groups using 7mg/ml and 5mg/ml were contrasted to the corresponding saline water treatment groups; it was found that the crude extract considerably increased the TFL reaction time. This is confirmed by the observation that drug effectiveness increases with the rate of tail withdrawal period away from heat stimulation also increased. If medication is more efficient, the skin of an animal will not respond to thermal stimulation (Strichartz, *et al*., 2009).

In the experimental mice, local anesthetic effects and anesthetic duration experiments of *D. stramonium* were evaluated by TFL test. The anesthetic effect results from TFL test exposed the extract of *D. stramonium* have a short lasting effect than the standard drug lidocaine2% solution, which showed anesthetic effect with a peak effect seen within 25 minute and anesthetic duration 20 to 45 min, and 25 to 35 min in the group treated with 7mg/ml and 5mg/ml crude extract, from the application. Whereas anesthetic effect of lidocaine2% started at 10 min and reach the peak effect at 25 min like the extract treatment group; and these experimental group was recovered gradually at long time (90 min) as compared to the test group of treatment. The leaf extract of *D. stramonium* induce anesthetic effect within the first 20 minutes and recovered sharply within few minutes as compared to the standard drug solution.

In this experiment the extract of *D. stramonium* began to expose anesthesia (induction time) at 20 min and coming back to normal life (recovery time) at 45 min is comparable with other studies conducted on the plant spilanthus (*spilanthus calva,and spilanthus oleraceae*) which showed that the anesthetic induction time at 20 min and recovery time is at 50 minute (Palpu, *et al*., 2010). This experiment also agrees with the study conducted on the same plant *datura stramonium* which had an induction time of 12 minute and recovery time at 28 minute (Adebayo and Olufayo, 2017). On the other side this experiment is disagree to the experiment demonstrated on Harsha ingection 22 which anesthetic duration is comparable to that of the standard drug lignocaine2% (85 minute), starting from 5min to 90 min (Suzuki, *et al*., 2019). This may be because of the secondary metabolites of *datura stramonium* which are used for anti cholinergic agent like alkaloids; troponoid, atropine and scopolamine are not as such denser like that of Harsha injection 22 compounds.

6. CONCLUSION AND RECOMMENDATIONS

Although synthetic anesthetics are commonly used, they are expensive and difficult to find locally and as a result, an option to synthetic anesthetic is plant anesthetic which is readily available, less expensive and more accessible. Thus, the use of natural anesthetics are chosen over synthetic ones because they are more environmentally and economically sustainable. The results from the current investigation showed that, in comparison to saline water treated groups, 70% ethanolic leaf extract of *D. stramonium* had high tail flick reaction time (9 sec at 25 min) in response to heat source whereas tail flick reaction time 3 sec to saline water throughout the experimental observation. Thus *D. stramonium* leaf extract should be utilized as local anesthetic because it has been shown to be positive of anesthetic effective when used at a dose of 5mg/ml and 7mg/ml in mice.

The following recommendations are forwarded to further research in-depth in light of the findings of the current study.

- In the study toxicity was no performed, it is recommended that dose toxicity should be conducted
- In this study, ethanol 70% was used as extraction solvent. It is recommended that the sample should be extracted other than ethanol solvent, fractionation should be performed on the anesthetic capacity of various solvent fractions.
- In this study, only leaf was used for the experiment and more study needs to be done on the other part of the plant especially the seed.

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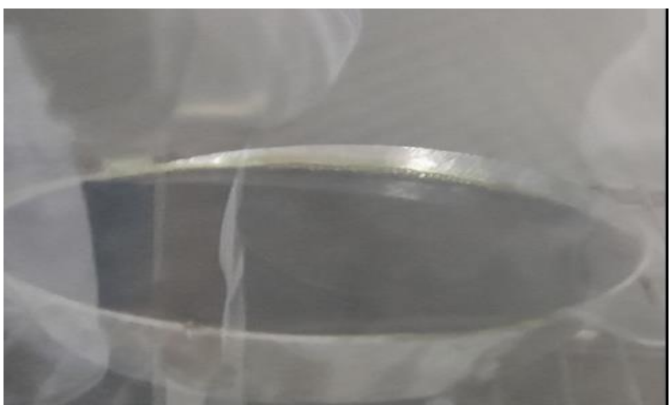
8. APPENDEX

A, *D.stramonium* at field, B, the dried *D.stramonium* and C, powdered *D. stramonium*



Crud leaf extract of *D. stramonium*

5mg/ml, 7mg/ml of leaf extract *datura stramonium* and lidocaine 2%

Figure 2 Measurement of tail flick reaction of *D.stramonium* extract at temperature of 55°C after subcutaneous administration was 9sec at 25 minute

D.stramonium extraction Administration of *D.stramonium* extracts

Ethanol (ethyl alcohol) and distil ter Orbital shaker