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By

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No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the Faculty of pharmaceutical sciences, The Superior University, Lahore in partial fulfillment of the requirements for the degree of Master of Philosophy in the field of “**Pharmacology**” in Faculty of pharmaceutical sciences at The Superior University, Lahore.

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DEDICATION

I dedicate this piece of work to:

Allah Almighty, my source of inspiration, strong pillar, and Muhammad (May Peace Be Upon Him) who enlightened the lives of billion, which aimed or bring success and prosperity to humanity.

My parents, my brothers, mentor, friends, relatives who shared their words of advice, whose love and encouragement infused the zest for struggle in my life and all my teachers who tried to show me the way to gain the knowledge

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LIST OF ACRONYMS

AE	Aqueous Extract
GIT	Gastro Intestinal Tract
Min	Minutes
ML	Milliliter
N. sativa	Nigella sativa
WHO	World Health Organization
ANS	Autonomic Nervous system
VGCC	voltage gated calcium Channels
ATP	Adenosine triphosphate
SR	Sarcoplasmic Reticulum
MLCK	myosin light chain kinase
EC	Excitation contraction Coupling
SRM	Selective receptors Modulator
GERD	Gastroesophageal reflux disease
IFFGD	International foundation for Functional gastrointestinal disorder
IBS	Irritable bowel syndrome
K	Potassium
ME	Methanolic extract
CO	Castor oil

ABSTRACT

Background: *Nigella sativa* (*N.sativa*) belongs to family Ranunculaceae is native to Iran, Turkey, Pakistan, India and many other countries. It has a special place in herbal medicines that are used in plant-based treatment of different ailments.

Aim: This study aims to investigate the potential antidiarrheal activity of the methanolic extract of *Nigella sativa* seeds in an experimental animal model of induced diarrhea.

Method: The study of *N. sativa* were collected, identified and authenticated with the help of a plant taxonomist. The seeds were dried at room temperature, grounded and passed through a 60 mesh sieve, and extracted with hydroalcoholic solvent. Adult albino rats weight between 150 and 200 gm of both sexes were used for experimental procedure. On the time of experiment, rats were randomly divided into five groups with six rats per group. Group 1 (negative control) receiving 10ml/kg in distilled water. Group 2 (Positive Control) receive 1mg/kg Loperamide & Group 3, Group 4, Group 5 (test groups) received three different doses such as; (100, 200, 300 and 400 mg/kg) of the crude extract and solvent fractions. All rats were given their respective therapeutic dose orally using oral gavage.

Results: At dose of *Nigella sativa* extract significantly diminish the GIT motility, no. of defecation and water content in the feces when compared with the standard drug Loperamide (1mg/kg) in a dose dependent manner (400mg/kg of *Nigella sativa* extract showed significant results) .

Conclusion: The study concludes that, result of this study confirms anti-diarrheal effect of *Nigella sativa* seeds provided the scientific basis for the traditional use of *N.sativa* plant as a treatment of diarrhea. The chemical constituents of methanolic extract *N.sativa* revealed the

presence of flavonoids, alkaloids and tannins which could be possible reason of this medicinal property.

Key words: Nigella sativa, charcoal meal, diarrhea, spasmolytic, gastrointestinal motility

CHAPTER NO. 1
INTRODUCTION

1-INTRODUCTION

1.1-Background

In human body, more than 600 muscles are present. The body makes ATP as to fuel a muscle, which muscles turns into mechanical energy. Body has three types of muscles tissue skeletal, cardiac, smooth muscles [1]. Muscles are composed of water, protein, inorganic salts, minerals, fats, carbohydrates. Skeletal muscle is multiple body functions, chemical energy converts into mechanical energy to produce energy, keep body position, move, maintains health. Muscle activation leads to result in muscle movements. It can be classified into 3 type's dynamic eccentric static, dynamic concentric, and Static muscle action is generate of force in the absence of a movement of limb or joint . Dynamic action is involving joint movement and force generation. Concentric muscle action is defined as shorten of muscle due to its origination and insertion come nearer to each other. Eccentric action defined as lengthening of the muscle because its origination and insertion move further than, causes decreased load from a bent elbow [2].

Cardiac muscles are striated, highly organized, structure distinct from that skeletal or smooth muscle. In which muscle contraction and relaxation are active processes [3]. Cardiac muscles are also called heart muscles, the layers of muscle tissue are present between the endocardium and epicardium. The cardiac muscle is made from sheet of cardiac muscle cells. These specialized cell junction and the arrangement of muscle cells enables cardiac muscle to contract quickly, forcing blood throughout the body. Cardiac muscles is controlled by the ANS (autonomic nervous system) [4].

Striated muscles are voluntary muscle also known as skeleton muscles, contract rapidly they are found attached to skeleton and soon undergo fatigue while on the other hand smooth muscles are

involuntary muscle ,contract slowly and do not undergo fatigue ,they are found in walls of internal organ .Latch bridge model in which two types of cross bridges ;phosphorylated normally cycling bridges and attached non phosphorylated slow cycling latch bridges [5].

Stomach and intestines help with ingestion, exist in urinary system it helps to rid the body of toxins and electrolyte balance. Its play a vital role in the regulation of blood pressure and tissue oxygenation. It originates from neural crest cell & mesoderm, different tissues contribute due to smooth muscles throughout the body. Smooth muscle in the body in which regulation of blood vessels play vital role for the development of neural crest cells [6].

Smooth muscles are involuntary muscles. These muscles are quite different from other types of muscles .It is usually found around tubes or ducts, wall of the intestine, airways blood vessels ,uterus etc. [7]. Smooth muscle cells are 3–10 μm thick and 20–200 μm long, in which nucleus is located in center, during contraction it takes a spindle shaped, smooth muscles cell are stagnant to the surrounding connective tissue by a basal lamina. It is found almost all organ system and plays important role in the duct of exocrine gland [8].

Smooth muscles contraction depends on calcium influx .There are two different types of calcium channels; a voltage dependent calcium channel, a receptor linked calcium channel [9]. The major route of contraction in smooth muscles rise in intracellular calcium channel, efflux of calcium channel release and reuptake into the Sarcoplasmic reticulum. In the plasma membrane the voltage gated calcium channels open to fluctuations in cell membrane potential and provided that important pathway for regulate entry of extracellular Calcium ions. VGCC (voltage gated calcium channels) are further classified as high voltage triggered and low voltage activated [10].

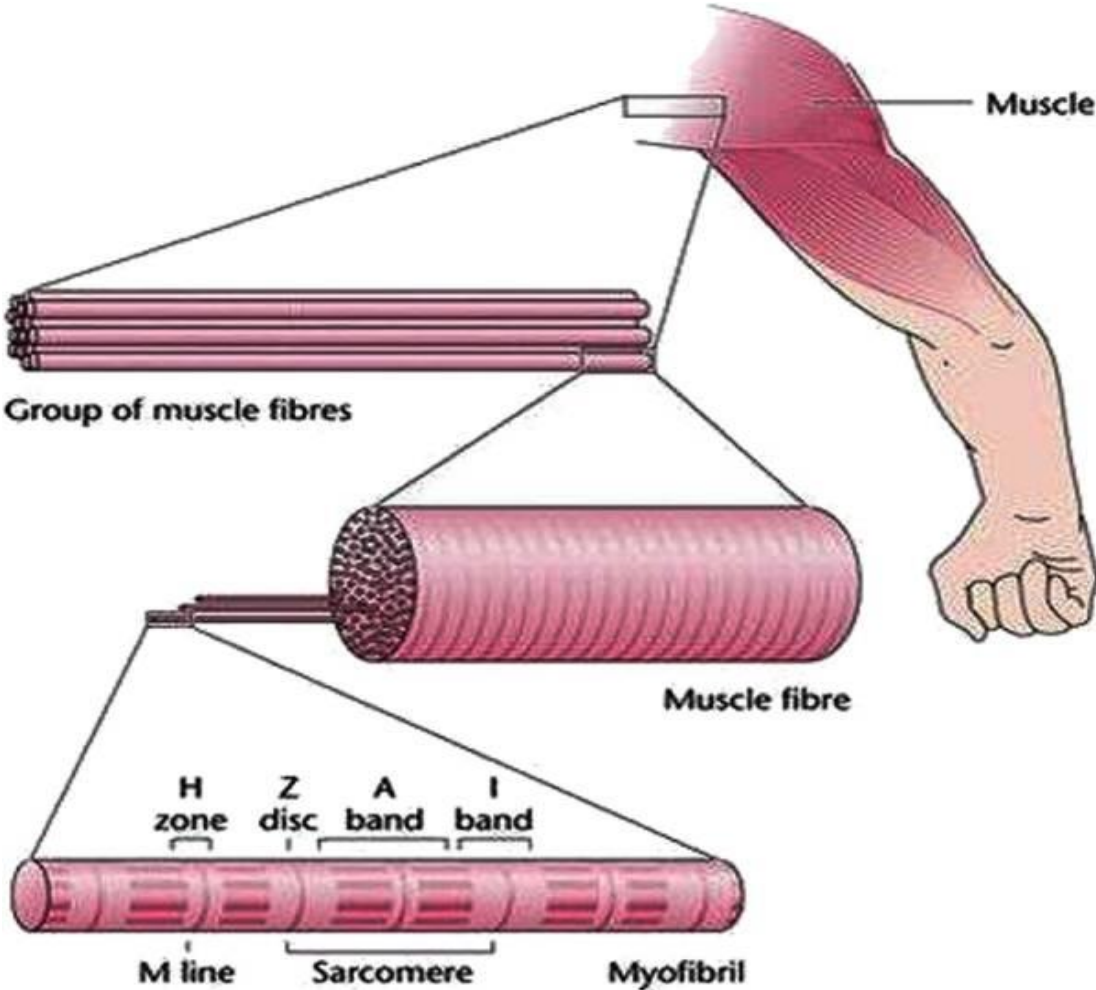
A defining feature of smooth muscle cells is their ability to contract. This property works as the excitable nature of these cells that allows for membrane potential dependent influx of calcium and the formation of cross bridges due to calcium dependent interaction between the myosin and actin [11]. Calcium ions affect all aspects of cellular function. It is a universal intracellular messenger that can participate in numerous biological processes in which muscle contraction, gene expression, cell growth and cell death. The disruption of calcium ion regulation often leads to cell dysfunction that can occur in life threatening diseases [12].

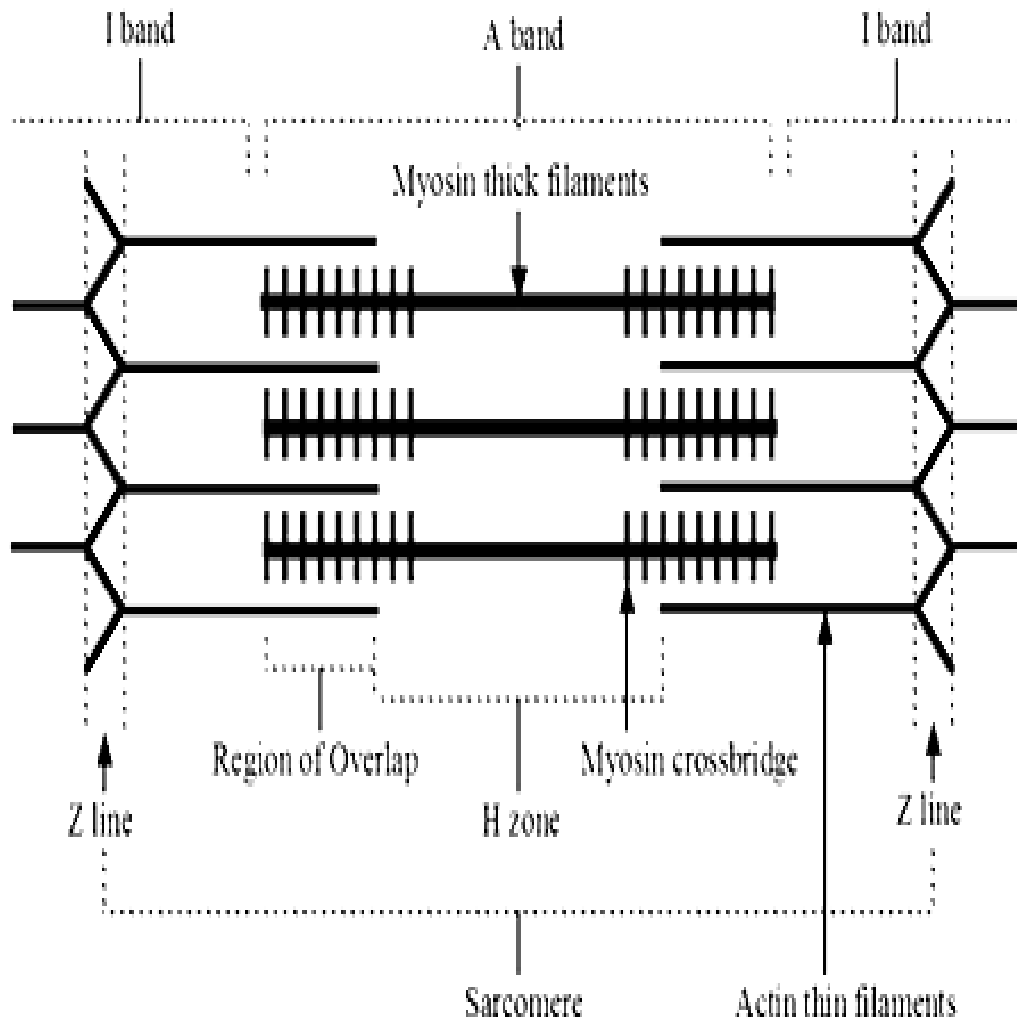
Actin and myosin both are found in almost all eukaryotic cells, require diverse cellular functions in which muscle contraction, cell motility, cytokinesis and organelle transport [13]. In muscles the type of myosin (myosin II) is the large protein consisting of two identical heavy chains, two pairs of light chains. Muscle contraction is the result of interaction between actin and myosin filaments that generates movements relative to one another [14].

Actin and myosin are present in large amounts in the smooth muscle cytoplasm. Myosin are the thick filaments and actin are the thin filaments. They both work together to generate force [15]. Myosin changes chemical energy into mechanical energy to release ATP, mechanical energy is used to slide actin filaments it can cause muscle fibers to contract & generate motion. The surrounding of actin & myosin filaments i.e. structure called SR (sarcoplasmic reticulum), is a network of tubules that reserve calcium ions [16].

Cytoplasm of muscle strands has elongated, fiber like structure called Myofibrils, each myofibril consists of repeating contractile units (sarcomeres). Z disc is present at the end of each sarcomere. It contains two different types of bands, A band is a dark colored band in which dense Myosin filaments and the I band is a lighter color and surrounds thin actin filaments [17].

Figure 1: Myosin and actin filaments in smooth muscles





[18]

Latch phenomena in which dephosphorylated myosin dissociates from actin very slowly and produce slow bridge cycle, maintained tension and tonic contraction occurred. Muscles cannot generate active tension but resist passive stretching. The main cause in which the myosin kinase and myosin phosphatase enzymes strongly activated ,the velocity of contraction increases .The lower activation of enzymes cause myosin head to remain attached to actin for longer time with less energy supply as ATP is required for detachment [19].

In all eukaryotic cell, calmodulin is the calcium modified proteins that remains a multifunctional intermediate calcium binding messenger protein indicate [20]. It increased the speed of contraction induced by the calcium ions, then developed maximum tension. Binding of calcium ions is required for the initiation of calmodulin because of it is an intracellular target .it acts as a part of calcium due to modifying its interaction of various target proteins i.e. kinases and phosphatases [21]. It is a regulatory component of myosin light chain kinase (MLCK) which in smooth muscle phosphorylates the regulatory light chain of myosin, a necessity for actin activated myosin ATPase activity [22].

Calmodulin plays important role in smooth muscle in which EC (Excitation contraction coupling) and in smooth muscles cross bridge cycling initiated that can causes smooth muscles contraction .On the other hand it can effect on contraction of muscles is by regulatory the movement of calcium ions in the cell & the SRM. [23].

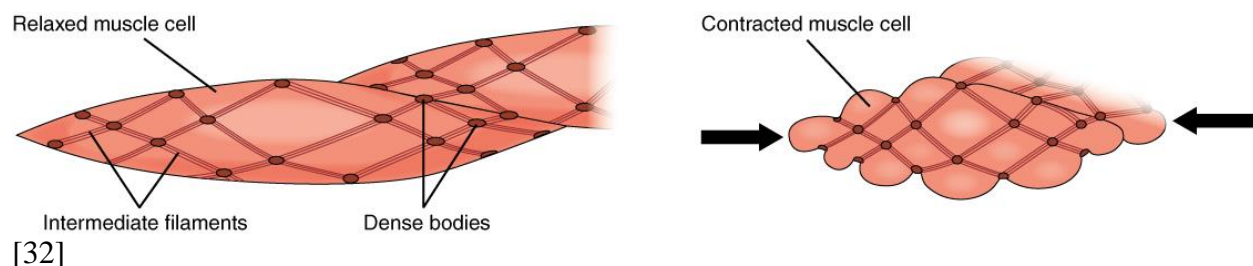
Smooth muscle are present in the wall of visceral organs, vasculature in the GIT tract (stomach esophagus, small and large intestine, sphincter, rectum) In the respiratory tract (trachea, bronchi, and bronchioles), female reproductive system (fallopian tube uterus, vagina), urinary tract (ureters, urinary bladder, urethra), hair follicles, iris of the eye [24].

There are two primary type of smooth muscles tissue that is single unit type muscle and multi-unit types muscles [25]. Phasic units are the single unit type of muscles in which allow slow and wave like contractions that are present in blood vessels, respiratory, urinary, digestive and reproductive tract, in which allows continuous contraction and more gap junction are occur, such as control the muscles of stomach [26]. Tonic units are the multi-unit type of smooth muscles in which lack of gap junction. These are occurred in large arteries, pulmonary passages, iris of eye. In the multiple unit type, individually each cells are contracted [27].

Spasm is involuntary contraction of the muscle, which is normally accompanied by pain [28]. Smooth muscles that are within the walls of hollow organ can go into spasm causing significant pain. This pain is like comes and goes is also called colicky [29]. Spasmolytic effect is the reduction of excessive smooth muscle contractility, responsible for cramping and discomfort in the abdominal area and caused by multiple conditions affecting the gastrointestinal (IBS), biliary or genitourinary tract. [30].

Muscles spasm are occurred due to insufficient blood supply, dehydration in which depletion of electrolytes in the muscles cells. Different symptoms that are occur during muscle spasm weakness of muscles ,numbness of skin, discomfort in the back ,neck and head ,feels like sensation of pins and needles, tremors, sleep issues, slow motions [31].

FIGURE 2: Represented relaxed and contracted smooth muscles.



GI tract problems are commonly affecting including the colon and rectum .most common examples are constipation irritable bowel syndrome, food poisoning, gas, bloating, GERD AND diarrhea. In the colon walls muscles are contract, in specific ways to move stool out of the body. Colon spasm are painful or cause digestive problems such as diarrhea [33]. IBS is the most common cause of colon spasm.it affects up to 45 million peoples in united states due to IBS according to IFFGD [34]. Four out of five people reported pain contributing to the severity of their IBS that is the most frequent factor [35].

Diarrhea is the second leading causes of death especially in children's. According to the age, 72% diarrheal disease are present in infants under two years, among children below five year of age [36]. colon spasm in which a spasm can occur sudden, spontaneous contraction of muscles. To treat involuntary muscles spasm, antispasmodic drugs, antidiarrheal, anticholinergics, antibiotics can be used. The normal value of water content in the stools nearly 10 mL/kg/d child and toddler, 200 g/d in the adolescent & mature because of imbalance in the small intestine and large intestine. Acute diarrhea in which onset of more than three loose stool in a day and not greater than fourteen days, except chronic & persistent diarrhea [37].

IBS (irritable bowel syndrome), also called spastic colon [38]. Due to colon contraction results in bloating, diarrhea, constipation and other digestive problem. IBS in which spontaneous contraction of muscles in small and large intestine, these contractions are spasms [39]. GIT motility disorder in which abnormal muscles movements and nerve contraction, motility disorder based upon the type of disorder in transit (delay, acceleration) [40].

Different ion channels plays important role such as ATP sensitive K channel, calcium ion activated K ions, Voltage sensitive calcium channels are expressed in GI tissues. In small intestine abnormal motility that can leads to symptoms of intestinal obstruction in the symptoms of pain, nausea, vomiting results weak contractions or from disorganized contraction [41]. Smooth muscles contraction are cause due to potassium ions concentration is higher than 30 mM (high K⁺) along opened of voltage dependent calcium channels, extracellular calcium ions move inward result in sustained contraction [42].

CHAPTER NO. 2

LITERATURE REVIEW

2-LITERATURE REVIEW

The traditional use of *Wrightia tinctoria* as an antidiarrheal and have antispasmodic effect, delay intestinal transit, gut motility suppress. It belongs to the Apocynaceae family. The active constituents that are present alkaloid, saponins, reducing sugar, tannins, flavonoids. The experimental model was used to induce diarrhea model of rats, isolated rat ileum to establish the therapeutic potential. The dose extract was used 500 and 1000 mg/kg dose inhibited the frequency and wetness of fecal dropping in castor oil induced diarrhea. *W. tinctoria* extract inhibited castor oil-induced diarrhea by reducing the number of deformed feces, frequency, and consistency of diarrhea in dose-dependent manner. Loperamide showed significant reduction in frequency of defecation and number of wet feces at 0.5 mg/kg dose [43].

The study was conducted on *Salvia officinalis* that can medicinally use as antidiarrheal. *S. officinalis* are commonly known as garden or red sage. In which the phytoconstituents are present volatile oil, flavonoids, diterpenes, 1, 8 cineol, camphor, borneol. At the dose of 100 and 300 mg/kg *N.sativa* extract block CO induced diarrhea in mice. These are indicate *S. officinalis* extract retains antidiarrheal activity mediate of voltage-dependent K^+ channels [44].

Z.Multiflora Boiss L is belonged to the Lamiaceae family .The phytochemical constituents are phenol, flavonoids, saponins, tannins, terpenes, thymol and carvacrol. *Z.Multiflora* and its component especially carvacrol, may have a calming effect on smooth muscle by inhibiting histamine and muscarinic receptors and calcium channel blocker [45].

These findings suggest (*P.emblica* fruit extract was anti-diarrheal property & reduce spasm), which may be mediated by a dual blockage (muscarinic receptors) and Ca channels, its usage as a remedy for diarrhea. *Pe.Cr* is commonly known as Indian gooseberry. Phytochemical

constituents contain (alkaloids, tannins, terpenes, flavonoids, sterols and coumarins) in these chemical constituents caused by inhibit of CO induced diarrhea, intestinal fluid accumulated in mice (at 500–700mg/kg) [46].

Several culture, plants of *Leea* genus are used traditionally to treat *diarrhea Leea aequata L.* leaf ethanolic extracts antidiarrheal properties in mice .The presence of flavonoids, alkaloids, tannins and phytosterols in the extract was detected using phytochemical screening and these compounds may be crucial to the antidiarrheal effect [47]. The extract was given to the positive control received Loperamide (3mg/kg) & Negative control received distilled H₂O (10ml/kg),the test groups received the extract in varying dosages (100,200 mg/kg).In a model of castor oil induced diarrhea ,the extract at 200 mg/kg considerably ($p<0.01$) reduced the overall number of bowel movements [48].

B.abbyssinica fresen used to treat diarrhea. The in vivo medicinal drug activity of *B. abyssinica fresen* extracts an solvent fraction castor oil induced diarrhea were tested in experimental model, enter pooling and antimotility test. After 1hr of dosing, 0.5ml of castor oil remained administered orally, then each mouse was placed transparent paper. Transparent paper was altered each 1 hr. intended for 4 hr. of observation, during the observation period, time of onset of diarrhea, number and weight of total and wet feces were measured. The crude extract, aqueous and chloroform fractions of *B. abyssinica* leaves have promising antidiarrheal effects, supporting the plants traditional use to treat diarrhea [49].

In this study to explain the excessive diarrhea occurrence and the high consequences of on the west side medication, the WHO is inspiring the exploration for conventional Chinese anti–diarrheal herbs, in which one of the herbs is (*Ophioglossum Vulgatum Linn*) family Ophioglossaceae which is historically apply on injuries & blisters, the popularity because the

extensive use of this medication in Taiwan. Here is rare research on its role on GI disease (anti-diarrheal and spasmolytic effect of the whole plant OV extract). Anti-diarrheal activity of EWOV doses orally intake 125, 250, and 500 mg/kg castor oil induced mice become assessed. Anti-diarrheal activity was assess to inhibiting the calcium ion channel [50].

Valeriana hardwickii is native to Pakistan, Burma, and Ceylon, antispasmodic and antidiarrheal activity. This plant is usually called as valerian. Vh.Cr exhibited dose-dependent (100– 300 mg/kg) protection against castor oil-induced diarrhea in mice. Loperamide i.e. antidiarrheal drug, similarly prevented the diarrhea. These data indicate the presence of CCB effect in the extract of *V. hardwickii* rhizome, probably facilitating its antispasmodic and antidiarrheal activities [51].

In this article to regulate the possible (anti-diarrheal and anti-spasmodic effects of methanolic extract of *Peganum harmala* L.). The seeds were dry in shade and crumpled into a coarse powder. Each of 100 g fine powders was extracted with 700 mL methanol by Soxhlet extraction, the crude MEPH was studied using the in-vivo castor oil-induced diarrhea model in mice, at 100 & 200 mg/kg showed 40% and 80% protection in mice while (positive control drug), Loperamide showed 100% protection. MEPH exhibit anti-diarrheal and anti-spasmodic effects, of its antimotility effect which were possibly, the inhibition of PDE enzyme [52].

To assess the overall (antidiarrheal activity of the 80% methanol extracts of *V officinalis* in mice). Group I and Group II (controls) received 10 mL/kg distilled water and standard drug (5 mg/kg Loperamide), whereas groups III, IV, and V (test groups) received 100, 200, and 400 mg/kg of the 80ME. The R-80ME at 200 mg/kg and 400 mg/kg significantly delayed the onset of diarrhea compared with (negative control). The charcoal meal test revealed that the R-80ME at 200 and 400 mg/kg produced a significant antimotility effect. The in vivo antidiarrheal index was resolute to be (95.25 at of 400 mg/kg) R-80ME [53].

2.1 - Nigella sativa

Nigella sativa is one of the high values in industry due to its beneficial effects on human health and its nutritional applications. It has a miracle herb on the basis of history and religion [54]. *Nigella sativa* also called black seed (kalonji). It belongs to (Ranunculaceae) family that is annual herb, small shrub with tapering green leaves and rosaceous white and purplish flower [55]. The ripe fruit contains numerous seeds, dark black in color[56]. The oil and seeds of *N. sativa* have been broadly utilized for a long time in the cure of different illnesses all through the world [57].

The seeds of *Nigella sativa* are small (1–5 mm). It produces (20–30 cm tall) with finely divided, rectilinear leaves. Flowers are pale navy and white in color with (10 petals). The constituents that are occur in *N. sativa* i.e., fats and fatty acids, essential oils, enzymes, protein, alkaloids, saponins flavonoids, phenols. Different extracts of seeds and active principles have been shown wide spectrum activities, may be single component effect or cumulative effects [58].

2.2-Pharmacological activities

This plant extensively used in natural remedy and the seed are used in spice, carminative, condiment, and aromatic. Traditionally the *N. sativa* used in diuretic, stomachic, liver tonic, digestion, diarrhea, also remove foul breathe, loss of appetite, vomiting, obesity, used in headache, migraine, antibacterial, anthelmintic [59].

N. sativa and its constituents are the remedies of varieties of diseases such as antioxidant, anti-inflammatory, antibacterial, antifungal, antiparasitic, antiviral, anticancer, gastrology, cardiology, and hepatoprotective and nephroprotective activities. The black seeds contain protein, fat, carbohydrates, alkaloids that isoquinoline alkaloids, terpenes, saponins, thymoquinine, Thymohydroquinine, dithymoquinone, p-cymene carvacrol, 4-terpineol, t-anethol,

sesquiterpene, α -pinene. *N. sativa* is safe and health promoter, especially observed in anti-fertility test [60].

Gastrointestinal disorders are usually stated in most part of the globe amongst i.e. Offspring. These disorders include (abdominal pain, irritable bowel syndrome (IBS), spasm, ulcerative colitis and constipation) & acute gastrointestinal disorder. GI disorders can lead to reduced life eminence and increased risk of anxiety and depression. Spasmodic compounds are dispersed in nature [61].

Antispasmodic drugs are composed with two group's i.e., anti-muscarinic or anticholinergic agents in which flavonoids and its derivatives from different medicinal plants and another is calcium channel blocker. Aqueous extract of the medicinal plants i.e. Aglycone flavonoids used to treat diarrhea and exhibited spasmolytic activity mediated through blockade of calcium channel [62].

2.3-Chemical Constituents

Constituents of *N. sativa* seeds are thymoquinine. The extract of *N. sativa* seeds contain many other constituents such as (proteins, vitamins, dietary minerals (such as Fe and Zn), crude fiber, alkaloids, carbohydrates saponins, steroid, p-cymene, limonene, terpenoids, and fatty acids). *N. sativa* seeds extract displayed antimicrobial activity with different efficacy against the tested pathogenic bacterial strains. *N. sativa* seeds contain a diversity of chemical components and functional groups related to their antimicrobial properties, it is natural precursors of nutraceuticals [63].

2.4 - (Table 1) Phytochemical Constituents of seeds of *N. sativa*

SR NO.	Constituents	Inference
1	Fixed oil and fats	+
2	Tannins	+
3	Proteins and amino acids	+
4	Alkaloids	+
5	Flavonoids	+
6	Steroids	-
7	Glycosides	+
8	Phenolics	+
9	Triterpenoids	+
10	Limonene	+

N. sativa has one of the amusing histories the seeds have been reported to use positive and helpful effects on dropping serum lipid profile, triglycerides level and attractive high-density lipoprotein levels. Black cumin is comprising mainly of (proteins, oil in addition to crude fiber and minerals. Carbohydrates, Iron, phosphorus, and calcium)have been reported to be at high levels while calcium, magnesium, zinc, copper, and manganese have been stated in lesser quantities [64].

Smooth muscle cells are contracted by initiation of receptors and mechanical stimulus (stretching) of contractile proteins. In the cell membrane, release of action potential causes the alteration in Membrane Potential or due to the initiation of stretch dependent ion channels in the plasma membrane, can lead to contraction. Medicinal plants induce contraction or relaxation of smooth muscles by many mechanisms involved interfering with (neurotransmitters, neuro-

mediators, second messengers, ionic channels, and other mechanisms). The seed extricate produces the spasmolytic effect by relaxing the ileum and this activity was mediated to the antagonist effect of calcium. The Volatile oil and ethanol extract Inhibited spontaneous movements of rabbit jejunum. The calcium channel blockade was suggested as spasmolytic mechanism [65].

The description of the phytochemical composition, antimicrobial activity of *N. sativa* seeds was approved using different extracts. *N. sativa* seeds extract oil was achieved by Soxhlet extraction method, absolute ethanol and hexane which are recognized to be appropriate when mining oil for testing biological activity. *N. sativa* seed oil have thymoquinine, avoid liposomes from suffering non-enzymatic lipid peroxidation. *N. sativa* seeds are often used to treat a wide range of (illnesses, including bronchitis, diarrhea, rheumatism, asthma and skin conditions). Used to stimulate the immune system, fight parasite infections, performance as a digestive aid and anti-diarrheal [66].

CHAPTER NO.3

METHODOLOGY

3-MATERIAL AND METHODOLOGY

3.1-Chemicals/reagents

Loperamide hydrochloride, castor oil, charcoal, distilled water, absolute methanol, and chloroform.

3.2-Equipments

Weighing balance, Freezer, Bloating paper.

3.3-Plant Collection

The seed of *N. sativa* were purchased from an Eco geographical area of Pakistan, Rahim yar khan. The crushed seed powder was dried at room temperature, ground and passed through a 60 mesh seize. Plant material was recognized and authentic by a plant taxonomist at the GCU Lahore (voucher number).

3.4-Preparation of extract

The *N. sativa* seed was shade dried then crushed and extracted with the ratio of 100g (1kg) by soaking the powder in 80 % hydroalcoholic solvent using (80% methanol; 20%distilled water) with daily shaking at room temperature for 12 days. The extract will then filter using Whatmann filter paper. The filtrate was then dried at room temperature. The crude extract was suspended in distilled water before administration to the animals. Stock solution of *N. sativa* extract by using distilled water and given to the animals according to the body weight in concentration of 100mg/kg, 200mg/kg, 300mg/kg. *N.sativa* extract was stored in an airtight flask in a refrigerator till further solvent fractionation and experimental procedure.

3.5-Animals:

Adult rats of both sexes, weighing among 150 and 200g were used. Animals remained providing with the animal feed and tap water .Before experimental procedure, food was reserved and water was allowed. In this study all animals were cared for in accordance with guidelines

3.6-Phytochemical screening

Nigella sativa extract was prepared freshly & exposed to standard phytochemical screening test for various chemical ingredients, saponins, alkaloids, glycosides, thymoquinine, , flavonoids, sterols.

3.7-Experimental Design

3.7.1-Anti-diarrheal activity

Rats be present kept in six cages containing 6 rats each. At the time of the experiment, the rats were randomly divided into five groups with six rats in each group. G1 (negative control) receiving 10ml/kg distilled water, G2 (positive control) getting 1mg/kg Loperamide and G3, G4, G5 (test groups) receiving different doses (100, 200, 300, 400 mg/kg) of the N. sativa methanolic extract and solvent portions. All rats remained given their respective treatment orally using oral gavage. Throughout the experiment, the rats were handled as per the global standard rules set for the Consideration and Utilization of Research center Creatures. In which dose dependent phenomena, in rats, all administrated doses was intraperitoneal. Rats were placed in cages linked with white blotting paper, 1 ml of castor oil was given orally to the rats 1hr after the above treatment. Paper was altered every hour after recording its weightiness. In laboratory, the filter paper was dried for the last 14 hrs and it was reweighted. Fecal water content was calculated. Rats were observed up to 5hr after administration of CO for the presence of diarrhea. Diarrhea,

which means watery (moist) unformed stool. The quantity of wet stool was counted consistently for 5hrs. [68]. In the control group the total number of wet stools was 100%.

3.7.2-Gastrointestinal motility

The gathering of rodents was abstained for 18 h yet could unreservedly admittance to water. Six group of six animal, groups A, B, C and D were administered charcoal suspension, 10% charcoal suspension 5% gum acacia were given orally 1h after castor oil treatment, 60 minutes later the animals were given intra gastrically *N.sativa* and group E treated with 2mg/kg Loperamide .Group F (control group) were treated with typical saline prior to getting the charcoal 40 minutes of the observation period, each rat was sacrificed and dissected. The small digestive tract was taken out, and all-out length (cm) was estimated. The charcoal movement of the pylorus is measured equally. The digestive charcoal travel is characterized as a level of the distance moved by charcoal to the complete length between the pylorus and the caecum.

CHAPTER NO. 4
RESULTS

4-RESULTS

4.1-Preliminary phytochemical analysis

Crude extract of *Nigella sativa* was evaluated. for the presence or absence of auxiliary metabolites like alkaloids, flavonoids, tannins, terpenoids, and steroidal compound, glycosides, phenols and saponins utilizing the methodology using the procedure described by Software [69].

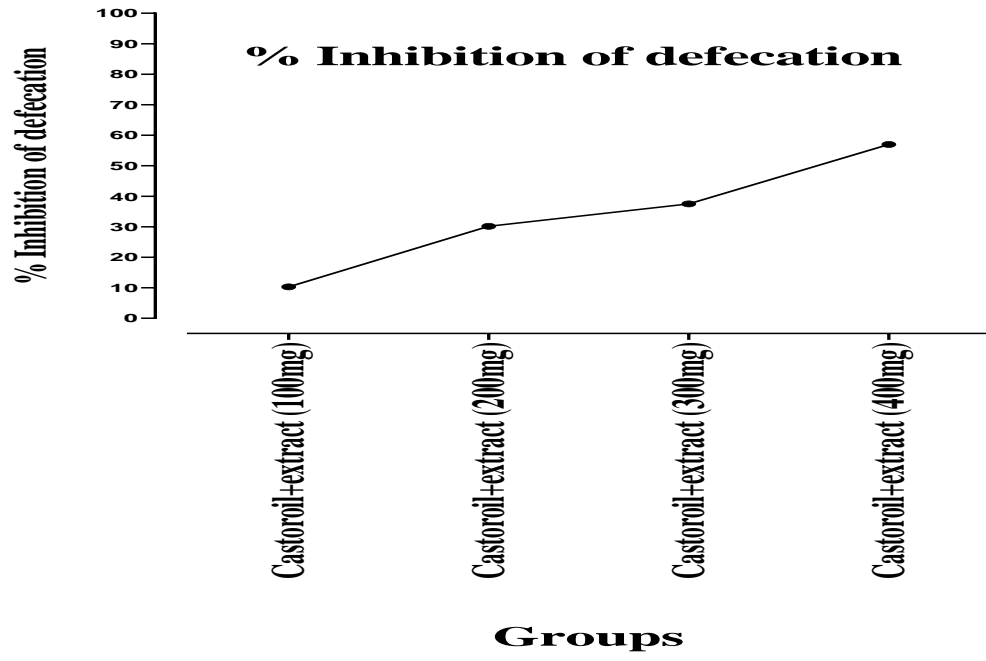
4.2-Castor oil induced diarrhea

The animals of control group apparent diarrhea in 30 min later administration of castor oil for the 4h. Loperamide 2mg/kg was largely eliminated by intraperitoneal injection (50.13%). Impact of the *Nigella sativa* separate was not generally as powerful as Loperamide at 100mg/kg, however in portion 200, 300, and 400 mg/kg. The concentrate of *Nigella sativa* created a dose dependent reduce in the no. of defecation over 4hr, ($p < 0.001$).

Table 4.1. Effect of methanolic extract of seeds of *N. sativa* on castor oil induced diarrhea in rats.

Treatment	Mean defecation in 4hr (g/kg body wt.)	% Inhibition of defecation
CO +N/S (2ml/kg)	24.62±0.36	0
Castoroil+loperamide(1mg/kg)	13.30±1.8**	50.13%
Castoroil+extract(100mg)	22.1±0.61*	10.3%
Castoroil+extract (200mg)	17.62±0.21**	30.18%
Castoroil+extract(300mg)	15.2±0.22**	37.5%
Castoroil +extract (400mg)	11.05±0.29**	56.99%

The methanolic extract was administered ip 1hr before CO administered, value are communicated as mean SEM from the research when contrasted with castor oil + saline treatment. SEM, standard error of mean, ** P<0.01, * P<0.001.



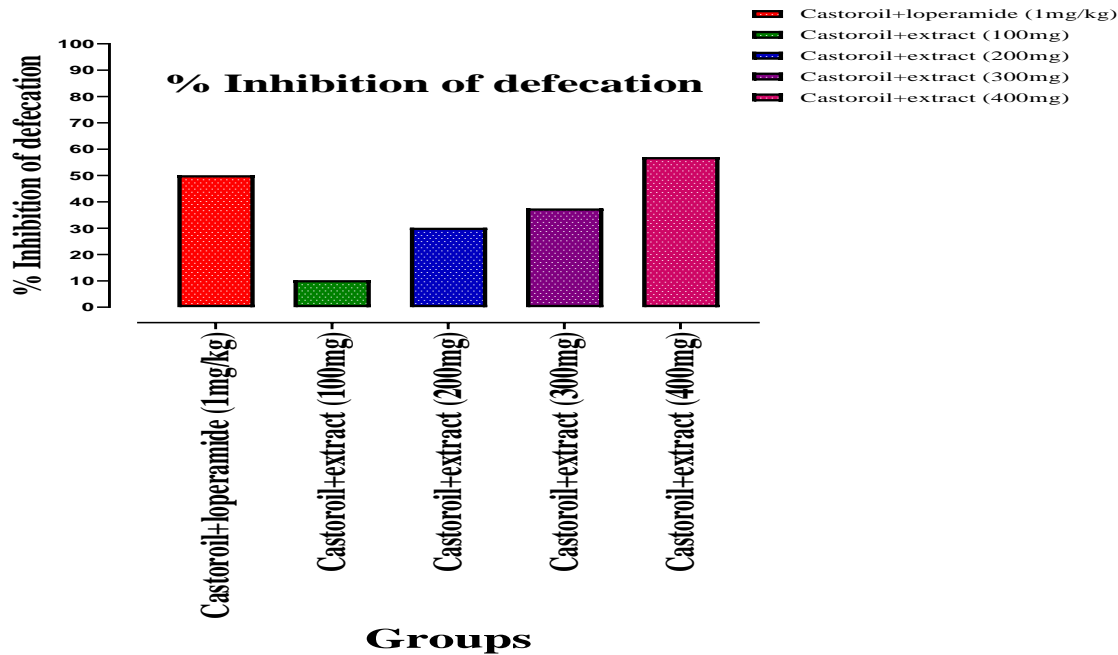


Figure 4: Graphical representation seeds of *N. sativa* on castor oil induced diarrhea in rats

4.3-GIT motility test

GIT motility was measured by calculating the distance (cm) travelled by charcoal. *Nigella sativa* reduced the gastrointestinal motility in a dose dependent manner. At dose of 400mg/kg it significantly reduced the GIT motility compared control group. Loperamide 1mg/kg caused a significant reduction in the propulsive movement and length of the intestine insulted by charcoal

$$T_0 - T_1 / T_0 \times 100$$

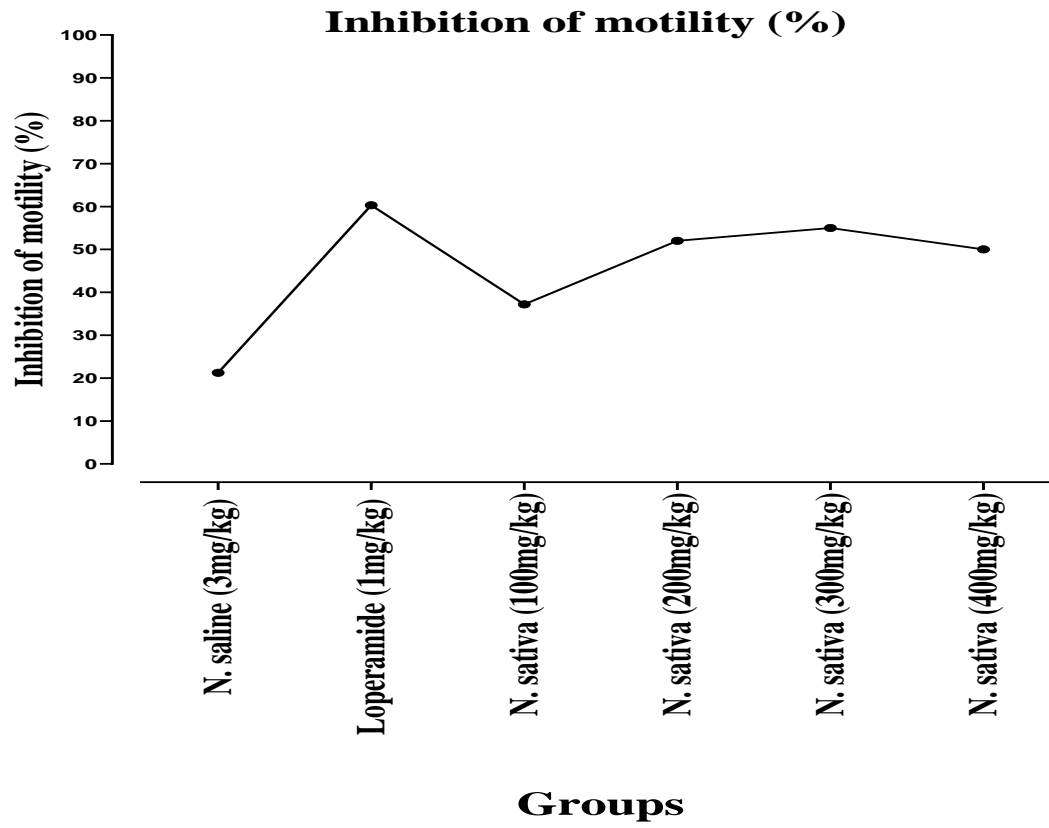
T_0 = length of intestine

T_1 = distance travelled by charcoal in intestine

Table 4.2. Effect of methanolic extract of seeds of *N. sativa* on gastrointestinal motility communicated as distance travelled by the charcoal suspension as percent of the absolute intestine length

Treatment	Dose	Total length of intestine(cm)	Distance travelled by charcoal(cm)	Inhibition of motility (%)
N. saline	3ml/kg	100.93±1.08	80.7±0.50	21.24
Loperamide	1ml/kg	107.58±2.8	41.928±3.10	60.3
N. sativa	100mg/kg	103±1.95	66.1±2.39	37.2
N. sativa	200mg/kg	104.3±3.50	52.3±5.34	52
N. sativa	300mg/kg	106.2±3.70	50.1±3.11	55
N. sativa	400mg/kg	108.72±4	44.08±3.5	50

Results are conveyed in mean ±SEM. Distance travelled by unpaired test followed by one way ANOVA. At dose of 400mg/kg it significantly reduced the GIT motility when it was compared with control group.



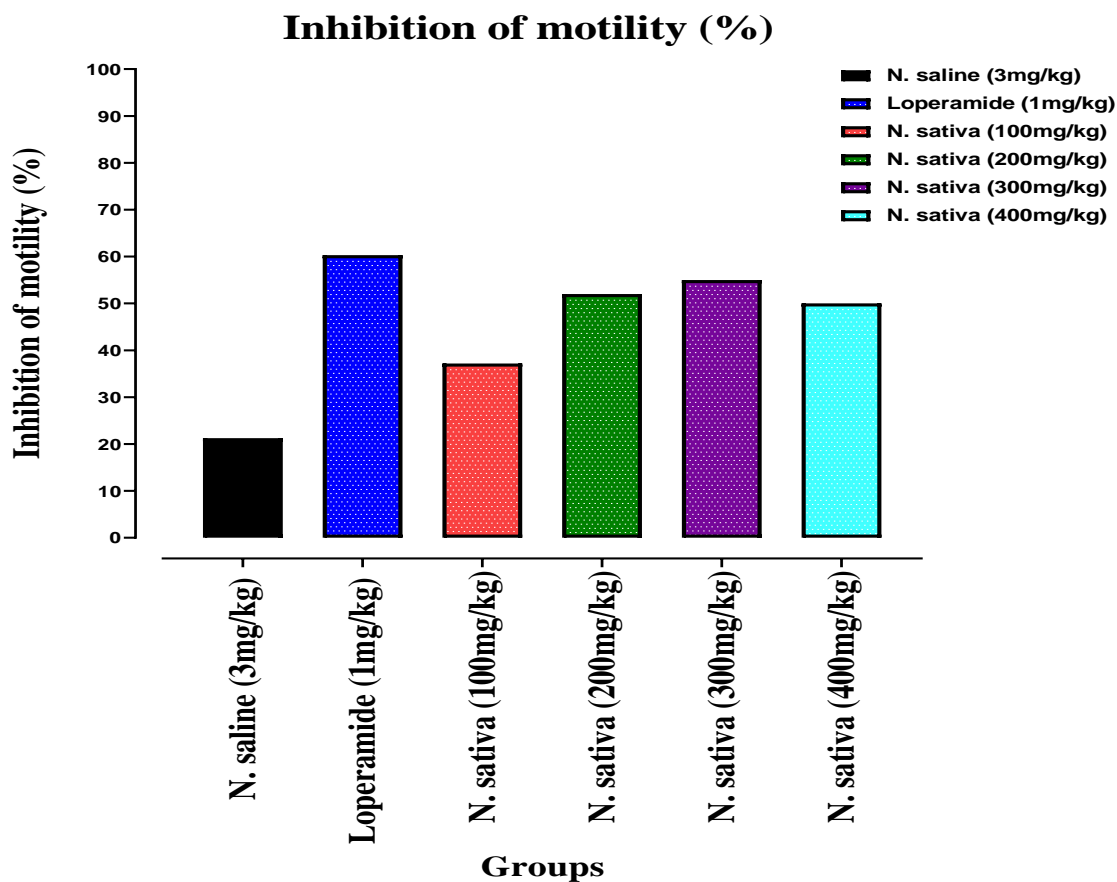


Figure 5: Graphical representation Effect of methanolic extract of seeds of *N. sativa* on gastrointestinal motility

Effect of particular oral dose of methanolic extract administer to mice

The acute toxicity study, all rats given the methanol extract stay alive. They seemed dynamic and healthy, without any indication no signs of unusual behavior

CHAPTER NO. 5

DISCUSSION

5-DISCUSSION

Individuals ordinarily utilize various part of plant for the treat sicknesses remembering diarrheal illness with no logical reason for their well-being and viability [70]. Moreover the study conducted on the methanolic extract of the seed of *N.sativa* seeds resulted in antidiarrheal effects against CO induce diarrhea in decreasing defecation frequency in rats. Consequently, this study was intended to assess the antidiarrheal impact of the 80ME seed extract of *N.sativa* , albino rats model beside CO induce GIT fluid accumulate as compared with the earlier study [71].

Castor oil consist of 90% ricinoleate, the active ingredient i.e. ricinoleic acid ,which has an inflammatory, irritated action on the mucosa layer of intestine therefore it can reduce prostaglandin, increment the penetrability of the mucosal cells and cause changes in the transport of electrolyte causing loose bowels. PG secretion *Nigella sativa* extract [72]. The standard drug i.e. Loperamide used for the positive control is a synthetic opiate agonist as a result in the myenteric plexus of massive gut activated μ opioid receptors [73]. Receptors occur presynaptic at the end parasympathetic cholinergic innervation of gastrointestinal smooth muscle which facilitatory affects smooth muscle contractility [74]. As a result, Loperamide (Standard drug) decrease individually delay fecal volume, delay fluid and reduce less electrolyte, causes increases faecal volume & majority viscosity discomfort [75].

Anti-diarrheal activity of the *N. sativa* extract followed mechanism:

a: *Nigella sativa* extract can proliferation the reabsorption of Nacl & water by reducing motility of intestine by charcoal food [76].

b: Alkaloids and flavonoids are inhibit prostaglandin, secretion induced by castor oil [77].

c: The chemical constituents of methanolic extract *N. sativa* in which the presence of flavonoids, alkaloids, and tannins. The methanolic extract of *N. sativa* reduce the diarrhea by increasing reabsorption of electrolyte and water on the standard drug like Loperamide [78].

Nigella sativa have a diarrheal effect due to found tannins, alkaloids, saponins, flavonoids, sterols, sesquiterpene, diterpenes, terpenes, flavonoids, terpenoids, these components are responsible from the biological activities of *N. sativa* extract. [79]. The extract of *nigella sativa* encounter the standards for the drugs acceptability as an antidiarrheal, these criteria include the formation of moist or unformed feces in animals and inhibition of GIT propulsive action of the GIT [80].

The successive excretion of stools of low consistency, because of the disturbance in water transport and electrolyte in the intestine known as diarrheal disease, different causes lead to diarrhea the mechanism involves in diarrhea. Osmotic diarrhea, in which increase intra-luminal osmotic solutions and decreased in absorption of water. Inflammatory diarrhea and infectious diarrhea, the viral, bacterial and pathogens disrupts of epithelium of the intestine, immune response to inflammatory condition in bowel [81]. During infectious diarrhea, the extract may contribute as an antidiarrheal activity. The *N.sativa* extract restrained unconstrained and agonist prompted compression of rodent ileum and decreased digestive motility. These effect contributed to the observed antidiarrheal activity [82].

In the diarrhea, abdominal pain is associated with changes in bowel habits and disrupts intestinal smooth muscle contraction these are common symptoms to produced diarrhea, to treat the diarrhea by selectively blocking voltage dependent Ca^{+} channels [83]. The contraction of

smooth muscle is dependent the concentration of intracellular Ca^{+2} . The 80ME of *N. sativa* inhibit the contraction of the rat ileum by blocking voltage dependent Ca^{+} channels, the extract caused a dose dependent (100, 200, 300, 400 mg/kg) relaxation of spontaneous contraction, due to Ca^{+} blockade. The crude extract of *N. sativa* seeds exhibits spasmolytic and antidiarrheal activity, inhibits the smooth muscle contraction, GIT motility to treat diarrhea [84].

The effect of *N. sativa* extract was similar to that of Loperamide. Therapeutic effect (standard drug) thought to remain antimotility and anti-secretory properties [85]. 80ME *N. sativa* extract, percentage defecation inhibition, wet fecal output weight, total stool output weight were detected in a dose-dependent manner [86].

Many countries, pharmacopoeias define a wide variety of plant species follows as remedies for abdominal cramps and diarrhea. Therapeutic studies of plants showed that they perform a combination of mechanisms more than one, Calcium channels block the opening K^{+} channels [87].

CHAPTER NO. 6

CONCLUSION

6-CONCLUSION

At the conclusion of this study, we support the claims of traditional healers regarding the use of methanol extract of *N. sativa* in the treatment of diarrhea. These findings concur with those of a foregoing investigation of *N. sativa* in terms of percent defecation protection for smooth muscles contraction inhibition. Oral administration of 80 M.E of *N. sativa* seed extract provided protection against CO induced diarrhea in albino rats, demonstrate notable delay in the onset of diarrhea. The wet stool frequency is bring down. The *N. sativa* seed extract demonstrate antimotility effect at its higher doses, so the study also showed the acute toxicity of the *N. sativa* plant extract i.e. found to be nontoxic, *N. sativa* make sure the safe use of the plant in folk medicine. We purpose to perform further studies on the mechanism of diarrhea at molecular level.

DECLARATION OF CONFLICTING INTEREST

The authors declare no conflicts of interest in this study.

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