

# Ethnopharmacological Study on *Nigella Sativa* Seeds Extracts: Pharmacognostical Screening, Abortifacient Potential and its Evolution Parameters

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## Abstract

*Current research investigates the feticide properties of Nigella sativa, a natural alternative to pharmaceutical feticides such as mifepristone. Abortion induced by mifepristone is often accompanied by distressing side effects, prompting the exploration of safer alternatives. Nigella sativa seeds were processed into coarse powder through grinding and subjected to ethanolic and aqueous extractions. Pregnant mice were administered extracts at 400 milligrams per kilogram of body weight, with a comparison group receiving 2.85 mg/kg p.o. of mifepristone. The study showed a significant decrease in fetal survival rates with nigella sativa extract treatment with the untreated control group exhibiting a 100% survival rate and the 400 mg/kg p.o. Dose groups showed rates of 26.66% for aqueous extract and 33.33% for ethanolic extract. Importantly, none of the fetuses in the mifeprin-treated group survived. The results indicate a promising 70% abortion rate with well-tolerated doses of Nigella sativa extracts, highlighting the potential for natural alternatives to mitigate the side effects associated with mifepristone administration. This research underscores the importance of exploring botanical sources for safer and more tolerable options in induced abortion.*

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**Keywords:** *Nigella sativa*, *Pregnancy*, *Prematurity*, *Newborn*, *Mortality*, *Drug effect*, *Drug Dose*, *Body Mass*, *Growth*, *Animal Model*, *Feticide*, *In-vitro*, *In-vivo study*.

## Introduction

Globally, plants account for about 25% of prescribed medications. There are several reasons why people are drawn to natural therapies. Sometimes, conventional medications show signs of inefficiency and abuse, which leads to the search for substitutes. Furthermore, the belief that natural products are safer has been reinforced by ecological consciousness and folk medicinal traditions. To further enhance their therapeutic potential, several medicinal plants are thought to interact via the immune system [1-2]. Black seeds are *Nigella sativa*. Its dry-roasted seeds are used in Indian cooking to add taste to curries, veggies, and pulses. The black seeds are like oregano and have a little bitter taste like mustard seeds. They are used in salad dressings, poultry recipes, and pod fruits. As it ages, the heavily ribbed and branching stem, which displays a spectrum of green hues from light to dark, may become hollow. *Nigella sativa* is well-known for its flexibility; it can grow in temperatures ranging from 5 to 25°C, with a preferred range of about 14°C. This resilient crop grows well in a variety of soil types, ideally in a pH range of 5 to 8 [3–4].

To allay worries about the negative effects and possible hazards connected with pharmaceutical feticides, the main goal of this study is to explore *Nigella sativa*'s possibilities as a natural feticide. It is crucial to remember that the seeds themselves are the main source of these nutritional advantages. Furthermore, 50% of PUFA and 22% of mono-unsaturated fatty acids are present in the fixed oil. Additionally, the overall polyphenolic content varies between 250 and 300 mg gallic acid per kilogram of oil. According to (GCMS), a variety of monoterpenes characterize the essential oil fraction (0.41 to 0.44%). About thirty-two chemicals have been found after a thorough examination. Moreover, thymoquinone, one of the bioactive ingredients in *Nigella* seeds, is linked to several pharmacological actions. These consist of cytotoxic, anthelmintic, analgesic, hepatoprotective, antidiabetic, anti-inflammatory, and antifertility qualities. Several plant-based chemicals also exhibit these properties [5].

A growing interest in natural therapies is reflected in the increasing global reliance on plant-based pharmaceuticals, which make up around 25% of prescribed drugs. This trend is motivated by factors such as the sometimes inefficiency and abuse susceptibility of traditional medicines. The assumption that natural goods provide a safer treatment option is further supported by the rich traditions of folk medicine and a growing ecological consciousness, which further reinforce this tendency towards alternative remedies. In this context, *Nigella sativa*, also referred to as black seeds, has garnered interest due to its many uses, especially in Indian cooking where the flavour of the dry-roasted seeds is enhanced in a variety of recipes. The plant's unique characteristics highlight its resilience, such as its highly ribbed and branching stem that can adapt to a wide range of temperatures and soil types [6].



thereby disrupting hormonal signaling pathways. Widely known as an essential component in medical abortion regimens, mifepristone's anti-pregestational activity contributes to its ability to induce pregnancy termination when used in combination with other agents like misoprostol. Its chemical structure, consisting of 29 carbon atoms, 35 hydrogen atoms, and 2 oxygen atoms, underscores its complexity and specificity in interfering with the progesterone hormone, making it a key pharmaceutical agent in reproductive healthcare [11-13].

### Dose

Mifepristone is a contraceptive that can prevent ovulation at a regular dosage of 2 mg per day. In the case of emergency contraception, a single mg taken before ovulation can delay it by 4- 5 days. Moreover, it can effectively induce medical abortion at a dosage of 600 mg, and a can also work as an emergency contraceptive.

### Natural Feticides

Before pharmaceuticals were developed, people used various non-pharmaceutical methods to induce abortions, including herbal and mineral preparations. It is difficult to assess their effectiveness because these practices predate clinical trials and the scientific method. Today, some claim that certain herbs and plants, available over the counter, can induce abortions alone or in combination with other doses and mixtures.

### Physiology

Pregnancy is usually divided into three trimesters, each lasting approximately three months. First, Second, Third, and Figure 1 are common examples of Natural Feticides [7].

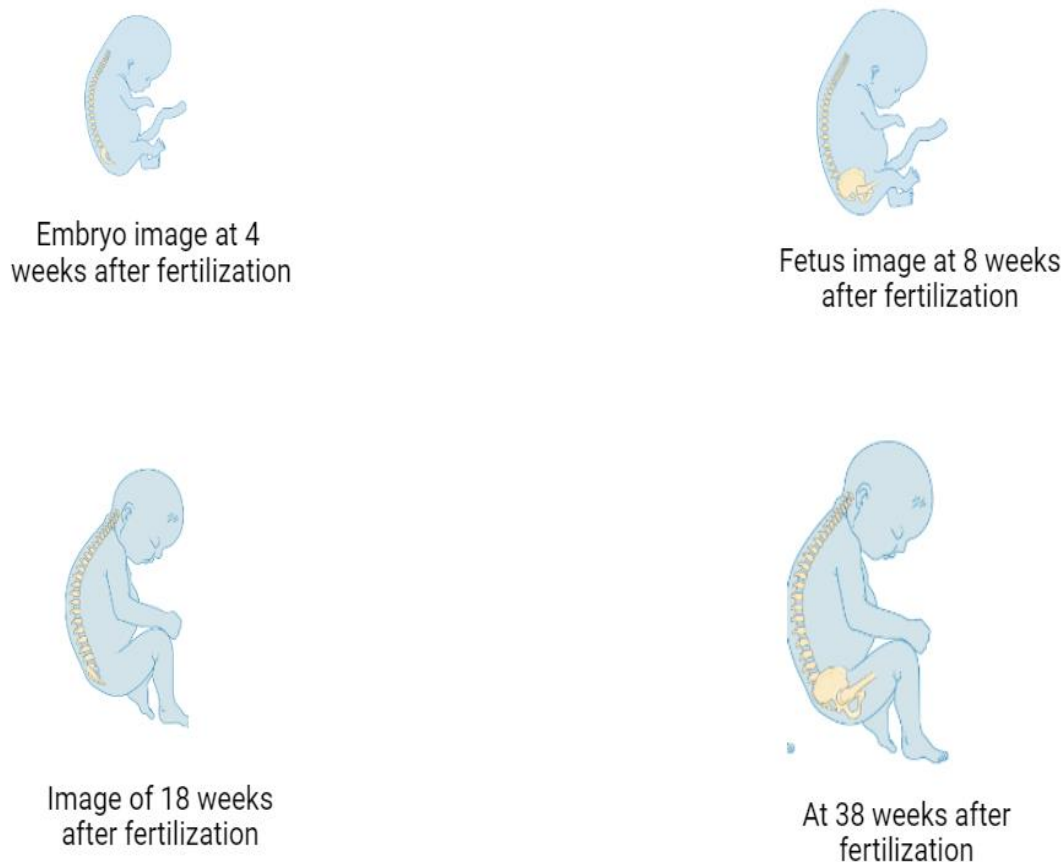


Figure 1: Fetal development stages.

## Material and Methods

### Chemicals material and preparation

All chemical products used in this study were obtained from the MRM College of pharmacy chemical store. Histamine (KP Laboratories), Chlorpheniramine Melate (KP Laboratories), Dexamethasone, DPPH (KP Laboratories), Ascorbic acid (CDH), Egg albumin (KP Laboratories), and all chemical reagents used for Pharmacognostical screening. Gathering information from peer-reviewed journals, based on the findings, it can be inferred that there is potential for further pharmacological research on *Nigella sativa* seeds [9].

### The process of gathering and verifying plants

The AV college of arts, science and commerce authenticated the plant number. We were assigned 222/Bot/AV College/3.4 after the final herbarium file was submitted to MRM Hyderabad.

### Size reduction of seeds

The seeds were ground into a coarse powder using a mixer grinder [9, 13]. The powder was passed through a No. 22 mesh to ensure consistency. After harvesting, store samples in an airtight container in a cool, dry place for analysis.

### Extraction of seeds

The extraction of powdered seeds was done using solvent extraction in an ethanolic solvent, and aqueous 250 gm of coarsely powdered seeds were packed into Soxhlet while in ethanolic soxhlation with ethanol in 1/3 ratio for 48 hr at 40-45°C. In aqueous solution with distilled water in a 1/3 ratio for 48 hr at 50-60°C. Whereas in the final extract was collected and dried at 30–40°C in both ethanolic and aqueous.



**Figure 2.1:** Soxhlet apparatus showing extraction.

### Seed Profile Characteristic

#### Screening of seeds

Externally black and internally white with a slight aroma. The taste is bitter, and the size is 2-3.5 x 1-2 mm.

#### Physiochemical analysis of seed powder

To determine the amount of moisture in a sample of the powdered drug, 10 grams were placed in tarred Petri dish [10]. the dish was placed in a 105°C hot air oven for one hour [11], and the dish with the dried powder was reweighed to calculate the loss on drying. The total ash value in the powdered drug, 5 grams, was accurately weighed and incinerated. After cooling, the ash-laden dish was considered to calculate the weight percentage (w/w) [12,15]. Mix 1 gram of ash with 25 ml of HCL to determine the Acid

**Insoluble Ash Value.** First, cover the crucible with a watch glass and boil the mixture for 5 minutes in a water bath. After cooling, rinse the watch glass with 5ml of hydrochloric acid and add it to the crucible.

Filter the mixture using a previously weighed filter paper, then dry the filtrate. To calculate the percentage of acid-insoluble ash, subtract the weight of the filter paper. Please let me know if you need any further clarification. Boil 1 gram of ash in 25ml distilled water for 5min to determine Water-Soluble Ash Value. Cover the crucible with a watch glass and place it in a water bath while boiling. After boiling, allow the mixture to cool. Rinse the watch glass with 5 mL of distilled water and add it to the crucible to calculate the water-soluble ash value., subtract the percentage of remaining content from 100% [12-13].

#### Foaming Index

A 500 ml conical flask was filled with 100 ml of water (4) with one gram of coarse powder to prepare the solution. The process began by placing the flask in a water bath and boiling it at a moderate temperature for 30 minutes (4) Once cooled down, the solution was carefully filtered into a 100 ml volumetric flask [14]. Water was added to the flask to dilute the solution to the required volume. The solution was then poured into a test tube and shaken lengthwise for 15 seconds to determine the foaming index. The test tube was then left undisturbed for 15 minutes, and the height of the foam was measured [13, 15-16].

#### Biological Evaluation

Antioxidant activity by DPPH free radical scavenging method [16-17]

##### Standard solution

To test the solubility of ascorbic acid, a solution was prepared by dissolving it in methanol. The resulting solution contained various concentrations of ascorbic acid, specifically 2, 4, 6, 8, and 10  $\mu\text{L}$ . This process was carried out as part of an experiment and was numbered 11 for reference purposes.

##### Test Solution

Stock solutions were prepared by dissolving 10 mg of dried ethanolic and aqueous extracts in 10 mL of methanol to obtain a 1 mg/mL concentration in both solvent systems.

##### DPPH Solution

50 mL of methanol was used to dissolve 4.3 mg of DPPH, which was then shielded from light with aluminum foil.

##### Estimation of DPPH

To assess a test sample's free radical scavenging activity, we mixed 50 ml of methanol with DPPH solution to measure the amount of light absorbed at a wavelength of 517 nanometers; a UV-visible spectrophotometer was used. This reading provided the

control data. Next, we screened the test sample at different volume levels (50, 100, 150, 200, 250 µL) and diluted it with methanol to yield 250 µL of each dose level. The resulting mixture was cut to the desired level with up to 50 ml of methanol. We added DPPH solution to each test tube and allowed it to react for 15 minutes. After the reaction, we measured the absorbance at 517 nm using methanol as a blank in a UV-visible spectrophotometer. We used a specific equation to calculate the percent antiradical activity (FRSA).

$$\% \text{ Antiradical activity} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

### Pharmacological Screening of Feticide Activity

#### Animal Study

Adult male and female virgin Swiss albino rats weighing between 180 and 200 grams. They were kept in controlled experimental conditions. Brands of Mifeprin 200 mg were used for the study. Acute Toxicity Study refers to evaluating the adverse effects of a substance or product when administered to living organisms for a short period. Study was conducted on Swiss albino mice, following the guidelines of OECD 423 (Organization for Economic Cooperation and Development). The experiment was carried out at MRM college of pharmacy, Telangana and the same institution provided the animals. Before the experiment, three mice were fasted overnight and given access to water. A total of fifteen mice were divided into five groups, each containing three mice. Using an intubation cannula, the groups were assigned different dosages of *Nigella sativa* seed extract (ethanolic and aqueous) via gavage. The experiment involved administering different doses of a substance to five groups of subjects. The study consisted of four groups of participants. The initial group was issued a dosage of 200 mg/kg of body weight, the second group was given 400 mg/kg of body weight, the third group was given 800 mg/kg of body weight, and the fourth group was assigned 1200 mg/kg of body weight. After evaluating the tolerability of the different doses, the researchers selected a 400 mg/kg body weight dose for both extracts for their feticide activity [13,15, 18].

**Table 1.1:** Grouping of Animals.

Animal Group No	Group Name
I	Control
II	Standard
III	Aqueous extract
IV	Ethanolic extract

Preparing the dosages for the extract



To prepare a 2% acacia suspension, 2 grams of acacia powder were accurately weighed and then suspended in 100 ml of 0.9% saline solution. To prepare a suspension with a concentration of 200 mg/ml, 4 grams of dried extract were added to 20 ml of the vehicle. The mixture was sonicated to form a homogeneous suspension. Both the ethanolic and aqueous extract suspensions were prepared similarly [13, 19].

### Pregnancy Preservation Animal Model

#### Procedure

Swiss albino rats of the female gender with regular estrus cycles were utilized to investigate the activity of feticides. smear was collected daily for 15 days by a dropper full of distilled water into the vagina, which was then collected back and placed on the slide after adding a drop of glycerin smear was examined under low-power microscopy (10X, 40X) to study different types of cells [13, 15,16]. The estrus cycle of rats has four phases, which the presence of certain types of cells can identify. If there are more white blood cells (leucocytes) present in the vaginal smear, then it indicates the dioestrus phase [20].

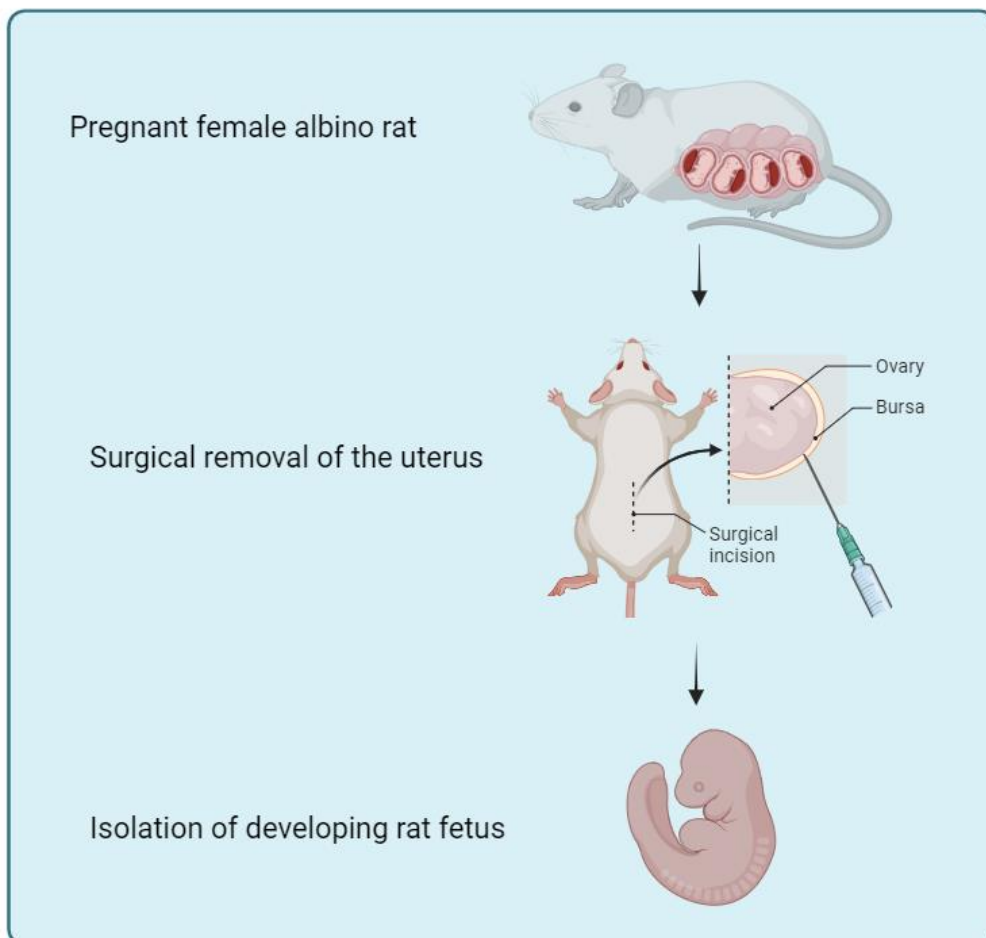
Four groups of rats with three regular estrus cycles were created each containing four rats. Group I control group, was given a vehicle containing only 1% Tween 80 orally. Group II was given mifepristone, a drug used as a standard, at a dosage of 2.85 mg/kg orally [13, 16, 19]. A 400 mg/kg dose of an aqueous extract was administered orally to Group III. The 400 mg/kg oral dose of Group IV ethanolic extract was administered. All rats were given their respective treatments from the 8th to the 20th day of pregnancy [13, 21-22]. On the 21st day, the rats were sacrificed under light ether anesthesia. We have gathered data on three factors: the weight of fetuses, the number of rats experiencing vaginal bleeding, and the percentage of abortions. To make the information easier to understand, we have organized it logically, starting with the most essential details. We have used simple, everyday language to improve clarity and avoid unnecessary jargon. In addition, we have preferred the active voice to make the text more straightforward and concise. The parameters of abortion were recorded. Here are the recorded data: weight of fetuses, number of rats with vaginal bleeding, and percentage of abortions [13, 24-25].

$$\% \text{ of Abortion} = \frac{\text{No. of Rat Aborted}}{\text{No. of Rat Used}} \times 100$$

$$\% \text{ of Survival} = \frac{\text{Live fetus}}{\text{Live fetus} + \text{Dead fetus}} \times 100$$



**Figure 2.2:** Preparation of vaginal smear of female albino rat.



**Figure 2.3:** Pregnant female albino rat and its fetus identification.

## Result and Discussion

### Features of *Nigella sativa* Seeds' morphology

Table describing the morphological characteristics. The seeds size  $2.5 \times 1.5$  mm and have a mild scent and flavor of bitterness. They are black on the outside and white on the inside [26–27].

### Screening for Phytochemicals

To identify different chemical ingredients, a phytochemical analysis was performed on a variety of *Nigella sativa* extracts.

### Intravitreal

The research highlights the important antioxidant qualities of the flavonoids and alkaloids present in *Nigella sativa* seeds, which are essential for preventing oxidative stress in the medical field.

**Table 1.2:** The seed characteristic features of *Nigella sativa* [13].

Sr. No.	Characters	Observations
1	Color	The exterior of the object is black, while the interior is white
2	Odor	Slightly aromatic
3	Taste	Bitter
4	Size	$2-3.5 \times 1-2$ mm

**Table 1.3:** Extraction of *Nigella sativa* seeds.

Sr. No.	Extract	Consistency of extract	Color of extract
1	Ethanolic	Semi-solid	Dark brown color
2	Aqueous	Semi-solid	Dark brown color

**Table 1.4:** Physiochemical analysis of *Nigella sativa* seeds [13].

Sr. No.	Analysis parameters	Observations % w/w
1	Loss on drying	3-4%
2	Total Ash value	4-5%
3	Acid insoluble ash value	0.19%
4	Water soluble ash value	1.30%
5	Foaming index	23 mL

### *Nigella sativa* seeds' antioxidant activity

Alkaloids have been shown to inhibit peroxidation, a process that can damage cells and be connected to several health issues. On the other hand, flavonoids oversee lipid peroxidation prevention, which is crucial for maintaining the integrity of cell membranes.

### Radical scavenging in DPPH

This work examined the DPPH radical scavenging properties of ethyl alcohol and aqueous extracts [13, 28–30]. The investigation's findings demonstrated the extracts' increased antioxidant and free radical-scavenging properties at higher concentrations. The DPPH activity of the plant powder extracts and ascorbic acid was assessed.

### Ascorbic Acid Comparison

Understanding the background is essential to understanding *Nigella sativa* extract's antioxidant properties. It was compared to ascorbic acid, a well-known antioxidant. The study found that water-based preparations and ethyl alcohol both exhibited potent antioxidant qualities comparable to ascorbic acid [31–32].

**Table 1.5:** Qualitative phytochemical analysis [13].

Sr. No.	Tests	Aqueous extract	Ethanollic extract
1	Carbohydrates		
	Result of Benedict’s test [13, 24] Result of Molisch’s Test [13]	(+) (+)	(+) (+)
2	Saponins [12]		
	By shaking the extract in a test tube [13]	(+)	(–)
3	Flavonoids [12]		
	Alkaline reagent test [13] Shinoda’s Test	(+) (+)	(–) (–)
4	Glycosides [12]		
	Baljet Test [13] Legal Test	(+) (+)	(+) (+)
5	Steroids and Sterols [12, 24]		
	Lieberman-Burchard Test Salkowski Test [13]	(+) (+)	(–) (–)
6	Tannins [12, 24]		
	with 5% ferric chloride solution	(+)	(–)
	with 10% lead acetate solution	(+)	(–)
	with 10% aqueous Potassium dichromate solution	(+)	(–)
7	Proteins and Amino Acids		
	Biuret Test [13, 24]	(+)	(+)
8	Alkaloids [12]		
	Dragendorff’s Test	(+)	(+)

	Mayer's Test	(+)	(+)
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(+) = Present, (-) = Absent

**Table 1.6:** Assessment of the effectiveness of Ascorbic Acid.

Sr. No.	Concentration(µg/ml)	Percentage inhibition (517 nm)
1	5	79.3
2	10	81.2
3	20	83.9
4	30	87.8
5	40	92.5
6	50	97.8

#### Acute oral study

Swiss albino mice were used in an acute oral toxicity investigation that followed OECD guideline 423. A dosage of 200–2000 mg/kg of extracts was administered to five groups of mice. No harmful side effects were observed, even when the dosage was increased to 2000 mg/kg by oral administration. This implies that extracts are safe. Further investigation into the feticide action of both extracts was decided upon, with a dosage of 400 mg/kg body weight [13, 19].

**Table 1.6:** Evaluation of Antioxidant activity.

Sr. No.	Concentration (µg/ml)	Percentage inhibition	
		Aqueous	Alcoholic
1	100	31.76	61.39
2	120	33.15	62.12
3	140	34.38	65.33
4	160	36.76	66.70
5	180	38.84	69.53
6	200	42.00	70.11

**Table 1.7:** Acute oral toxicity study Ethanollic and Aqueous extract of *Nigella sativa* seeds extract.

Sr. No.	Dose in mg/kg (B.W.) P.O. (EENS)	Dose in mg/kg (B.W.) P.O. (AENS)	Observation
1	200	200	All Animals Survived

2	400	400	All Animals Survived
3	800	800	All Animals Survived
4	1200	1200	All Animals Survived
5	2000	2000	All Animals Survived

EENS stands for Ethanolic extract of *Nigella sativa*, while AENS stands for Aqueous extract of *Nigella sativa*.

### Pharmacological Screening of Feticide Activity

Both extracts were given to female albino rats to test for their feticide properties.

This study evaluated the feticide efficacy of both drugs in combination with mifepristone. The quantity and weight of viable fetuses fell, although feed and water consumption remained unchanged, according to the study [13, 19, 31–32]. Treatments of mifepristone (400 mg/kg, p.o.) were administered to the groups. The body weight of both extracts contained only dead fetuses. The survival rate of fetuses in the control group was 100%; in the animals that received both extracts, this percentage fell to 26.66% and 33.33%, respectively. None of the fetuses in the Mifepristone group survived [13, 33–34]. The study's findings demonstrated that Mifepristone had a 100% success rate in inducing an abortion, while the groups treated with ethanolic and aqueous extract had success rates of 50% and 75%, respectively. Abortion incidents and vaginal hemorrhage are correlated. The study found that extracts from *Nigella sativa* and mifepristone both have feticide qualities, which decreased the number of viable fetuses and ultimately resulted in abortion [13, 35].

This work investigates the complicated dynamics of the feticide effects in female albino rats produced by Mifepristone and *Nigella sativa* extracts, which significantly advances the field of reproductive health research. The noted decrease in the number and weight of viable fetuses in pregnancy indicates the efficacy of these medications in assisting with pregnancy termination. The absence of viable fetuses in the groups given 400 mg/kg of *Nigella sativa* extracts orally highlights the potential efficacy of these organic chemicals in disrupting the normal course of pregnancy [13, 36].

**Table 1.8:** Albino female rats were used for feticide effects of aqueous and alcoholic extracts.

Sr. No.	Evaluation Variable	Aqueous extract	Alcoholic extracts	Mifepristone
1	Fetus weight (g)	0.26 ± 0.25**	0.41 ± 0.31*	0.0 ± 0.0**

2	Aborted rat (No.)	4	2	4
3	Aborted rat (%)	75	50	100
4	Fetuses dead (No.)	1,4,2,5	4,5,2,2	0,0,0,0
5	Live fetuses (No.)	0,0,0,5	2,3,0,0	0,0,0,0
6	Vaginal bleeding in rats (No.)	4	2	4
7	Fetus survival ratio (%)	26.31	41.25	0

Data presented as mean  $\pm$  SEM. Checked if there was a significant difference between groups by using ANOVA. There was statistical significance if \*P < 0.05 or \*\*P < 0.01 compared to the control group using Dunnett's t-test. Each group had four samples. Standard: Mifepristone (2.85 mg/kg), Extract (400mg/kg)

Significant new information regarding the relative effectiveness of herbal and pharmaceutical feticides is provided by the notable decline in fetus survival rates, particularly in the groups treated with extract and mifepristone. The 100% survival rate of the control group which received no treatment illustrates the remarkable impact of the medications administered on fetal viability. The observed differences in survival rates between treatment groups serve as motivation for further investigation to clarify the mechanisms by which mifepristone and *Nigella sativa* extracts induce pregnancy [13, 33–34].

The study's noteworthy component is the variation in abortion induction success rates. Mifepristone showed a 100% success rate, however the groups that used ethanolic and aqueous extracts had success rates of 50% and 75%, respectively. This variance calls for further investigation into the subtle pharmacological effects of these compounds and possible ways in which they can interact with the intricate mechanisms regulating the preservation of pregnancy. The correlation between abortion episodes and vaginal bleeding provides a physiological context for the obtained results, hence indicating the need for more research to determine the precise mechanisms by which these drugs induce pregnancy termination.

The study provides insight into the relative safety profiles of Mifepristone and *Nigella sativa* seed extracts, with an immediate focus on abortion induction. Interestingly, the constant intake of feed and water in all groups, regardless of treatment, suggests that there are no appreciable negative impacts on these vital physiological markers. This data is essential for assessing the overall safety and tolerability of these medicines, especially when considering possible therapeutic uses [37–38]. As a feticide, *Nigella sativa*, a naturally occurring botanical source, offers a fresh perspective on reproductive health research. *Nigella sativa's* growing range and soil pH tolerance demonstrate its

adaptability to a variety of environmental situations, which lends an ecological perspective to its prospective application in reproductive health therapies. This plant's resilience underlines even more how viable it is as a readily available, sustainable resource for medicine.

## Conclusion

The results of this investigation demonstrate the feticide qualities of the extracts of *Nigella sativa* and mifepristone. The increasing percentage inhibition at various concentrations (5 µg/ml: 79.3%, 10 µg/ml: 81.2%, 20 µg/ml: 83.9%, 30 µg/ml: 87.8%, 40 µg/ml: 92.5%, 50 µg/ml: 97.8%) indicates the concentration-dependent response, which offers important information about their possible efficacy. Furthermore, this research advances our knowledge of the fundamental mechanisms of action and safety profiles of these compounds. The observed differences in successful attempts caused by the live fetuses not being present in the extract-treated groups highlight the necessity of continued research to identify the precise chemicals causing these effects. Furthermore, the study highlights *Nigella sativa's* effective as a sustainable substitute for traditional pharmacological feticides, given its ecological resilience. This understanding of the natural extract's effectiveness promotes more research into its potential uses in therapies for reproductive health. In the conclusion, the current study advocates for a complete approach to reproductive health that includes both pharmaceutical and natural alternatives, laying a critical foundation for future research initiatives.

### Ethical Permission

From Institutional Animal Ethical Committee registration number 11195/b/03/CPCSEA MRM College of Pharmacy, Hyderabad, Telangana India

### Conflict of Interest

Authors declare no conflict of interest.

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