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

Mariam K. Alamoudi¹, Khulood A. Almehmadi², Md Sajid Ali³, Sana Hashmi⁴, Mohammed Fulayyih Essa Alharbi⁵, Mohammad Rashid⁶, Md Tanvir Athar⁷, Shamshir Khan⁸*, Samiuddin Khaja⁹*, Naseem Akhtar¹⁰

Abstract

Current research investigates the abortifacient properties of Nigella sativa, a natural alternative to pharmaceutical abortifacients 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 mifepristone-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.

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

¹ Department of Pharmacology and Toxicology, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia
² Department of Pharmacology and Toxicology, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia
³ Department of Pharmaceutics, College of Pharmacy, Jazan University, Jazan, 45142, Kingdom of Saudi Arabia
⁴ Department of Pharmaceutics, Unaizah College of Pharmacy, Qassim University, Unaizah, 51911, Qassim, Saudi Arabia.
⁵ Buraidah Central Hospital, Al Taalim, Buraydah, 52361, Qasim, Kingdom of Saudi Arabia
⁶ Department of Pharmacognosy and Pharmaceutical Chemistry, College of Dentistry and Pharmacy, Buraydah Private Colleges, Buraydah-51418, Al-Qassim, Saudi Arabia
⁷ Department of Pharmacognosy and Pharmaceutical Chemistry, College of Dentistry and Pharmacy, Buraydah Private Colleges, Buraydah-51418, Al-Qassim, Saudi Arabia
⁸ Department of Pharmacognosy and Pharmaceutical Chemistry, College of Dentistry and Pharmacy, Buraydah Private Colleges, Buraydah-51418, Al-Qassim, Saudi Arabia
⁹ Department of Pharmacognosy and Pharmaceutical Chemistry, College of Dentistry and Pharmacy, Buraydah Private Colleges, Buraydah-51418, Al-Qassim, Saudi Arabia
¹⁰ Department of Pharmacology, MRM College of Pharmacy, Ranga Reddy-501510, JNTUH Hyderabad, Telangana, India, samiuddinkhaja121@gmail.com
Introduction

Approximately 25% of prescribed drugs are plant-based globally. This attraction to natural remedies arises from various factors. Conventional medicines, at times, exhibit inefficiency and susceptibility to misuse, prompting the exploration of alternative solutions. Additionally, folk medicine traditions and ecological awareness have fostered the perception that natural products offer a safer choice. Moreover, many medicinal plants are believed to interact with the immune system, contributing to their therapeutic potential [1-2]. Nigella sativa is black seeds. In Indian cuisine, its dry roasted seeds serve as a flavorful addition to curries, vegetables, and pulses. The black seeds, resembling oregano with a subtle bitter undertone akin to mustard seeds, find their way into various culinary applications, including pod fruits, vegetables, salads, and poultry recipes. The stem is highly ribbed and branched, exhibiting a range of green hues from light to dark, and may be hollow as it ages. Known for its adaptability, Nigella sativa can thrive under a wide temperature range, from 5 to 25°C, with a specific range of around 14°C. This hardy crop can successfully grow in various soil types, typically with a pH range of 5 to 8 [3-4].

In this study context primary objective is to investigate Nigella sativa potential as a natural abortifacient, aiming to address concerns surrounding the side effects and potential risks associated with pharmaceutical abortifacients. It's important to note that these nutritional benefits come primarily from the seeds themselves. Additionally, the fixed oil consists of 22% mono-unsaturated fatty acids and 50% PUFA. Moreover, the total polyphenolic content ranges from 250 to 300 mg gallic acid, which is equivalent to per kg of oil. The essential oil fraction (0.41 to 0.44%) is characterized by a combination of monoterpenes, as determined by (GCMS). A comprehensive analysis has identified nearly 32 compounds. Furthermore, the bioactive components in Nigella seeds, such as thymoquinone, are associated with various pharmacological activities. These include hepatoprotective, antidiabetic, anti-inflammatory, antifertility, anti-oxytocic, cytotoxic, anthelmintic, and analgesic properties. These activities are also found in other plant-based compounds [5].

The increasing global reliance on plant-based pharmaceuticals, constituting approximately 25% of prescribed drugs, reflects a growing interest in natural remedies driven by factors such as the occasional inefficiency and misuse susceptibility of conventional medicines. This inclination towards alternative solutions is further bolstered by the rich traditions of folk medicine and an escalating ecological awareness, fostering the belief that natural products offer a safer therapeutic choice. Amidst this landscape, Nigella sativa, commonly known as black seeds, has gained attention for its versatile applications, particularly in Indian cuisine where its dry roasted seeds enhance the flavour of various dishes. The plant's distinctive features, including a highly ribbed and branched stem with adaptability to a broad temperature range and diverse soil types, underscore its resilience [6].

In the current study, our principal aim is to explore the potential of Nigella sativa as a natural abortifacient, addressing concerns surrounding side effects and potential risks associated with pharmaceutical abortifacients. It is imperative to underscore that the nutritional benefits of Nigella sativa primarily emanate from its seeds. The fixed oil composition, comprising 22% mono-unsaturated fatty acids and 50% polyunsaturated fatty acids (PUFA), adds to its nutritional profile. Additionally, the seed's total polyphenolic content, ranging from 250 to 300 mg of gallic acid per kg of oil, contributes to its overall health. The essential oil fraction, characterized by combining monoterpenes through gas chromatography-mass spectrometry (GCMS) analysis, reveals a diverse profile of nearly 32 compounds. Beyond its nutritional composition, Nigella sativa seeds harbor bioactive components such as thymoquinone, showcasing a spectrum of pharmacological activities, including hepatoprotective, antidiabetic, anti-inflammatory, antifertility, anti-oxytocic, cytotoxic, anthelmintic, and analgesic properties. These multifaceted activities align with broader trends observed in other plant-based compounds, emphasizing the potential of Nigella sativa as a valuable natural resource in therapeutic research [6-8].
Pharmaceutical Abortifacients

Pharmaceutical abortifacients play a significant role in the field of pregnancy termination. Prostaglandin analogs, including misoprostol and gemeprost, are commonly employed to terminate pregnancies, typically up to 24 or 60 days of gestation. It is worth noting that the efficacy of misoprostol is higher when administered vaginally compared to oral administration [9-10].

Mifepristone

Mifepristone, commonly referred to as RU-486, works by blocking the progesterone receptor. Mifepristone also known as Mifepristone is a derivative of estrane progestins with a molecular weight of 429.5.

![Mifepristone Chemical Structure](attachment:mifepristone.png)

Mifepristone, with the chemical formula C_{29}H_{35}NO_{2}, is a synthetic steroid compound that belongs to the class of antiprogestogens. This molecule plays a crucial role as a progesterone receptor antagonist, effectively blocking the binding of progesterone and thereby disrupting hormonal signaling pathways. Widely known as an essential component in medical abortion regimens, mifepristone's anti-progestational 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 [11-12].

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 Abortifacients

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 Abortifacients [7].
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, 14]. 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 1: Fetal development stages.
Ethnopharmacological Study on Nigella Sativa Seeds Extracts: Pharmacognostic Screening, Abortifacient Potential and its Evolution Parameters

Pharmacognostical Screening

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 [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 μL. 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 aluminium 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 Abortifacient 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 abortifacient activity [13,15, 18].
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 [18-19].

The Pregnancy Maintenance Animal Model
Procedure
Swiss albino rats of the female gender with regular estrus cycles were utilized to investigate the activity of abortifacients. 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 glycerine smear was examined under low-power microscopy (10X, 40X) to study different types of cells [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 [15-16]. 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 [21-22]. On the 21st day, the rats were sacrificed under light ether anaesthesia. 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].

No. of Rat Aborted

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

Live fetus

\[
\% \text{ of survival} = \frac{\text{Live fetus}}{\text{Live fetus} + \text{Dead fetus}} \times 100
\]
Figure 2.2: Preparation of vaginal smear of female albino rat.

Figure 2.3: Vaginal smear slide that displays the diestrus phase.
Ethnopharmacological Study on Nigella Sativa Seeds Extracts: Pharmacognostic Screening, Abortifacient Potential and its Evolution Parameters

Figure 2.4: Vaginal smear slide showing proestrus phase.

Figure 2.5: A slide of a vaginal smear indicating estrus phase.
Figure 2.6: A slide of a vaginal smear showing the metestrus phase.

Figure 2.7: A vaginal smear slide is a sample of cells taken from the vagina that is used to examine for any abnormalities. This slide shows the presence of sperm.
Figure 2.8: Pregnant female albino rat that, displaying the presence of fetus in her uterus.

Figure 2.9: Surgical removal of the uterus and removal of the amniotic sac.
Figure 2.10: Developing fetus is linked to the placenta through the umbilical cord.

**Result and Discussion**

**Morphological characteristics of Nigella sativa Seeds**

Table outlining the morphological features. The seeds are externally black, internally white with a slight aroma and bitter taste; measures 2-3.5 × 1-2 mm [26-27].

**Phytochemical screening**

Various extracts of Nigella sativa were subjected to phytochemical investigation to determine various chemical constituents.

**In-Vitro**

The study emphasizes the significant antioxidant properties of alkaloids and flavonoids found in Nigella sativa seeds, which are crucial for combating oxidative stress in healthcare.

**Table 1.2: Pharmacognostical screening of Sativa seeds.**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Color</td>
<td>The exterior of the object is black, while the interior is white</td>
</tr>
<tr>
<td>2</td>
<td>Odor</td>
<td>Slightly aromatic</td>
</tr>
<tr>
<td>3</td>
<td>Taste</td>
<td>Bitter</td>
</tr>
<tr>
<td>4</td>
<td>Size</td>
<td>2-3.5 × 1-2 mm</td>
</tr>
</tbody>
</table>
Table 1.3: Extraction of Nigella sativa seeds.

<table>
<thead>
<tr>
<th>No.</th>
<th>Extract</th>
<th>Color of extract</th>
<th>Consistency of extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanolic</td>
<td>Dark brown color</td>
<td>Semi-solid</td>
</tr>
<tr>
<td>2</td>
<td>Aqueous</td>
<td>Dark brown color</td>
<td>Semi-solid</td>
</tr>
</tbody>
</table>

Table 1.4: Physiochemical Analysis of Sativa seeds [13].

<table>
<thead>
<tr>
<th>No.</th>
<th>Analysis parameters</th>
<th>Observations w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Loss on drying</td>
<td>4 %</td>
</tr>
<tr>
<td>2</td>
<td>Total Ash value</td>
<td>4.82</td>
</tr>
<tr>
<td>3</td>
<td>Acid insoluble ash value</td>
<td>0.15 %</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble ash value</td>
<td>1.71 %</td>
</tr>
<tr>
<td>5</td>
<td>Foaming index</td>
<td>24 ml</td>
</tr>
</tbody>
</table>

Antioxidant Activity of Nigella sativa seeds

Alkaloids are shown to inhibit peroxidation, a process that can lead to cell damage and contribute to various health issues. On the other hand, Flavonoids are responsible for inhibiting lipid peroxidation, a vital aspect of maintaining cell membrane integrity.

DPPH Radical scavenging

In this study, ethyl alcohol and aqueous extracts were tested for their DPPH radical scavenging activity [13, 28-30]. The study revealed that the higher concentration of extracts led to increased free radical scavenging activity and antioxidant properties. DPPH activity was compared among both extracts of plant powder and ascorbic acid.

Comparison with Ascorbic Acid

In order to better understand the antioxidant properties of Nigella sativa extract, it is important to provide some context. It was compared to ascorbic acid, a well-known antioxidant. The study found that both ethyl alcohol and aqueous extracts exhibited a high degree of antioxidant ability, comparable to ascorbic acid [31-32].

Table 1.5: Qualitative Phytochemical Analysis.

<table>
<thead>
<tr>
<th>No. Sr.</th>
<th>Tests [12]</th>
<th>Ethanolic extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrates</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td></td>
<td>Result of Molisch’s Test [13]</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td></td>
<td>Result of Benedict’s test [13, 24]</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>2</td>
<td>Tannins [12, 24]</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td></td>
<td>with 5% ferric chloride solution</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td></td>
<td>with 10% aqueous Potassium dichromate solution</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td></td>
<td>with 10% lead acetate solution</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>3</td>
<td>Alkaloids [12]</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td></td>
<td>Dragendorff’s Test</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td></td>
<td>Mayer’s Test</td>
<td>(+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>
Table 2.5: Assessment of the effectiveness of Ascorbic Acid

<table>
<thead>
<tr>
<th>No. Sr.</th>
<th>Concentration (µg/ml)</th>
<th>Percentage inhibition (517 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>79.3</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>81.2</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>83.9</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>87.8</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>92.5</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>97.8</td>
</tr>
</tbody>
</table>

Acute oral study

An acute oral toxicity study was conducted on Swiss albino mice according to OECD guideline 423. The mice were split into five groups, each receiving 200 to 2000 mg/kg of extracts. No signs of toxicity were observed even when the dose was increased up to 2000 mg/kg via oral administration. This indicates that extracts are safe. For further studies on the abortifacient activity, a dose of 400 mg/kg body weight was selected for both extracts.

Table 2.6: Evaluation of Antioxidant activity.

<table>
<thead>
<tr>
<th>No. Sr.</th>
<th>Concentration (µg/ml)</th>
<th>Percentage inhibition</th>
<th>Aqueous</th>
<th>Alcoholic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>30.76</td>
<td>60.39</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>32.15</td>
<td>62.12</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>140</td>
<td>34.38</td>
<td>64.33</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>160</td>
<td>37.76</td>
<td>66.70</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>180</td>
<td>39.84</td>
<td>68.53</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>200</td>
<td>42.00</td>
<td>70.11</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.7: Acute oral toxicity study Ethanolic and Aqueous extract of Nigella sativa seeds extract

| No. Sr. | Dose in mg/kg (B.W.) P.O. (EENS) | Dose in mg/kg (B.W.) P.O. (AENS) | Animal in each group | Observation
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>200</td>
<td>3</td>
<td>All animals survived</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>400</td>
<td>3</td>
<td>All animals survived</td>
</tr>
<tr>
<td>3</td>
<td>800</td>
<td>800</td>
<td>3</td>
<td>All animals survived</td>
</tr>
<tr>
<td>4</td>
<td>1200</td>
<td>1200</td>
<td>3</td>
<td>All animals survived</td>
</tr>
<tr>
<td>5</td>
<td>2000</td>
<td>2000</td>
<td>3</td>
<td>All animals survived</td>
</tr>
</tbody>
</table>

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

Pharmacological Screening of Abortifacient Activity

Female albino rats were administered both extracts for abortifacient effects. This study evaluated the abortifacient potential of Mifepristone and both extracts. The study revealed a decrease in the number and weight of live fetuses, while feed and water intake remained unchanged [31-32]. The groups treated with Mifepristone and the 400 mg/kg, p.o. The body weight of both extracts had no live fetuses, only dead ones. The survival rate of fetuses decreased from 100% in the control group to 26.66% and 33.33% in animals administered both extracts, respectively. None of the fetuses survived in the Mifepristone group [33-34]. The study found that Mifepristone had a 100% success rate in inducing abortion, while the success rates for the aqueous extract and ethanolic extract groups were 75% and 50%, respectively. The episodes of abortion are associated with vaginal bleeding. The study showed that both Mifepristone and both extracts of Nigella sativa had abortifacient effects, resulting in a decrease in the number of live fetuses, ultimately leading to abortion [35].

In the realm of reproductive health research, this study contributes significantly by delving into the intricate dynamics of abortifacient effects induced by Mifepristone and Nigella sativa extracts in female albino rats. The observed decrease in the number and weight of live fetuses across treated groups underscores the potency of these agents in facilitating pregnancy termination. The absence of live fetuses in the groups treated with the 400 mg/kg oral dose of Nigella sativa extracts highlights the potential efficacy of these natural compounds in disrupting the ordinary course of pregnancy [36].
Table 2.8: Albino female rats were used for abortifacient effects extracts.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Control</th>
<th>Standard (Mifeprizone - 2.85 mg/kg)</th>
<th>Aqueous extract 400mg/kg</th>
<th>Ethanolic extracts 400mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No live fetuses are found in the individual rat</td>
<td>10,11,9,9</td>
<td>0,0,0,0</td>
<td>0,0,0,4</td>
<td>3,3,0,0</td>
</tr>
<tr>
<td>2</td>
<td>No dead fetuses</td>
<td>0,0,0,0</td>
<td>0,0,0,0</td>
<td>0,5,2,4</td>
<td>5,4,2,1</td>
</tr>
<tr>
<td>3</td>
<td>No rat aborted</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Weight of fetus (g)</td>
<td>1.28 ± 0.03</td>
<td>0.0 ± 0.0**</td>
<td>0.27 ± 0.27**</td>
<td>0.48 ± 0.27*</td>
</tr>
<tr>
<td>5</td>
<td>Percentage aborted</td>
<td>0</td>
<td>100</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>Survival ratio of Fetus (%)</td>
<td>100</td>
<td>0</td>
<td>26.66</td>
<td>33.33</td>
</tr>
<tr>
<td>7</td>
<td>No. of rats with vaginal bleeding</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Data presented as mean ± 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.

The noteworthy decline in the survival rate of fetuses, particularly in the Mifepristone and extract-treated groups, provides critical insights into the comparative effectiveness of pharmaceutical and botanical abortifacients. The fact that the control group, which remained untreated, exhibited a 100% survival rate emphasizes the stark impact of the administered substances on fetal viability. This divergence in survival rates among treatment groups sets the stage for further investigations into the underlying mechanisms through which Mifepristone and Nigella sativa extracts exert their abortifacient effects [33-34].

An intriguing aspect of the study lies in the differing success rates of abortion induction. While Mifepristone demonstrated a 100% success rate, the aqueous and ethanolic extract groups exhibited success rates of 75% and 50%, respectively. This variation prompts an exploration into the nuanced pharmacological actions of these substances and their potential interactions with the complex processes governing pregnancy maintenance. The identification of vaginal bleeding as a correlate to abortion episodes adds a layer of physiological understanding to the observed outcomes, warranting further investigation into the specific pathways through which these agents instigate pregnancy termination.

With an immediate focus on abortion induction, the study sheds light on the comparative safety profiles of Mifepristone and Nigella sativa extracts. Notably, the consistent feed and water intake across all groups, irrespective of treatment, indicates a lack of significant adverse effects on these essential physiological parameters. This information is crucial in evaluating these agents' overall tolerability and safety, particularly in the context of potential clinical applications [37-38]. Nigella sativa, a natural botanical source, as an abortifacient, represents a novel dimension in reproductive health research. The adaptability of Nigella sativa to various environmental conditions, as highlighted by its cultivation range and soil pH tolerance, adds an ecological perspective to its potential utilization in reproductive health interventions. The hardiness of this plant further emphasizes its viability as a sustainable and readily available resource for medicinal purposes.
Conclusion

The findings of this study highlight the abortifacient properties of both Mifepristone and Nigella sativa extracts. The demonstrated concentration-dependent response, as illustrated by the increasing percentage inhibition at different concentrations (5 µg/ml: 79.3%, ten µg/ml: 81.2%, 20 µg/ml: 83.9%, 30 µg/ml: 87.8%, 40 µg/ml: 92.5%, 50 µg/ml: 97.8%), provides valuable insights into their potential efficacy. Additionally, this research contributes to a deeper understanding of the underlying mechanisms of action and safety profiles associated with these substances. The observed variations in successful endeavours led by the absence of live fetuses in the extract-treated groups emphasize the need for ongoing investigations to pinpoint the specific compounds responsible for these effects. Moreover, considering the ecological adaptability of Nigella sativa, the study suggests its promising role as a sustainable alternative to conventional pharmaceutical abortifacients. This insight into the natural extract's efficacy encourages further exploration of its applications in reproductive health interventions. Ultimately, the present study lays a crucial foundation for future research endeavours, advocating for a comprehensive reproductive health approach encompassing pharmaceutical and natural alternatives.

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.

Acknowledgement

This study is supported via funding from Prince Sattam bin Abdulaziz University project number (PSAU/2023/R/1445).

References


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


30. Antioxidant and antiproliferative activity of glycosides obtained by.pdf.


