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PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION FOR THE ABORTIFACIENT POTENTIAL OF THE SEEDS OF *NIGELLA SATIVA* L.

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ABSTRACT
Nigella sativa L (Ranunculaceae family) or commonly known as black cumin has long been used as a phytomedicine for antidiarrhea, appetite enhancer, diuretic, antibacterial, analgesic, anthelmintic, various skin diseases, anti-inflammatory, back pain, hemiplegia (paralysis of the hands or feet) and rheumatism. The objective of this study was to investigate pharmacognostical, phytochemical features, antioxidant activity and abortifacient effects of ethanol and aqueous extracts of *Nigella sativa* seed by using DPPH assay method and pregnancy maintenance model respectively. The different pharmacognostical parameters were evaluated as per standard protocols with some modifications. The preliminary phytochemical studies were performed with various reagents and chemicals on seed extracts in order to determine the various secondary metabolites. The ethanol and aqueous extracts of *Nigella sativa* seeds were screened for *in-vitro* antioxidant activity by oxygen radical scavenging such as 1, 1-diphenyl-2- picrylhydrazyl (DPPH) method. Abortifacient activity was studied in rats at 400 mg/kg doses administered orally from 8thto 20thdayof pregnancy. Rats were laparotomised on 21st day. Weight of fetus, percentage of abortion and thelive and dead fetuses were observed in both horns of the uterus. Pharmacognostical studies reveal the macroscopical characters of *Nigella sativa* seeds. Phytochemical screening of the ethanol and aqueous extracts of *Nigella sativa* seeds revealed the presence of secondary metabolites like alkaloids, carbohydrates, proteins and amino acids, tannins, saponins, glycosidesand flavonoids. The ethanol and aqueous extracts showed good dose dependent free radical scavenging property. IC_{50} values for aqueous and ethanol extracts were calculated by DPPH method. Aqueous extract have shown more free radical scavenging power as compared to ethanol extract. Ascorbic acid was used as standard. In this experiment, abortion is induced by using pregnancy maintenance model. In contrast, no live foetus was recorded in the animals treated with mifepristone as well as the 400 mg/kg, p.o. body weight of the ethanolic and aqueous extract having live foetus. Whereas ethanolic and aqueous extract having dead foetus and there was no dead foetus in the mifepristone treated animals. The ethanolic and aqueous extract of *Nigella sativa* at doses of 400 mg/kg has abortifacient effects, supporting its uses in traditional medicine. Further studies on the isolation of bioactive phytoconstituents of *Nigella sativa*seeds and their mechanism of action are strongly recommended before its application to humans.

Keywords: *Nigella sativa*, Pharmacognostical, Phytochemical features, Antioxidant activity, Abortifacient effects.

1. INTRODUCTION
One of the fundamental areas of human life is fertility and conception. Rapid rise in population has caused serious problems in the economic growth and all-round human development in countries like Nigeria, leading to poverty. Family planning has been promoted through several methods of contraception and abortion such as surgical intervention, drugs like synthetic steroidal contraceptives (e.g. mifepristone, misoprostol), prostaglandins and antiprogestins, but they are often marked with serious side effects such as gastrointestinal problems, severe and painful uterine contractions, systemic illness, permanent sterility or even death [1]. Therefore, the screening of plants with abortifacient activity and the subsequent identification and characterization of the active principle(s) will be a useful

guide towards the formulation of cheaper, affordable contraceptive with reduced toxicity [2]. Artificial antioxidants such as butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA) are commonly reported for their efficiency in delaying cell deterioration but are also suspected to have negative health effects like carcinogenesis and toxicity [3]. Therefore, replacement with natural antioxidants could reduce health risks. *Nigella sativa* is a traditional and natural source of antioxidants. In fact, *Nigella sativa*is capable of free radical inhibition and can also significantly reduce oxidative stress [4]. *Nigella sativa*, or black seed, is an annual flowering plant of the Ranunculaceae family, which is native to the Mediterranean and the neighboring countries of Pakistan and India. Although the plant is not a significant component of the human diet, in the Middle East it is incorporated into the way of life and the daily diet [5] as a spice and preservative [6]. Black seed has been widely used for thousands of years to treat a variety of diseases and medical conditions, including asthma, high blood pressure, diabetes, inflammation, cough, headache, eczema, fever, dizziness, and influenza [6-8]. Over the last five decades, numerous scientific studies have affirmed the pharmacological qualities of *Nigella sativa* seeds and demonstrated its anti-inflammatory, antibacterial, antihistamine, antidiabetic, anticancer, and antihypertensive activity [6]. Extracts of *Nigella sativa* contain volatile and non-volatile oils, amino acids, proteins, carbohydrates, alkaloids, nitrogen compounds, saponins, and minerals such as sodium, calcium, iron and potassium; overall, more than 100 compounds have been isolated from black seed and their structure elucidated [5, 6]. Many studies have attributed the bulk of the pharmacological activity of *Nigella sativa* to its quinone content, which includes thymoquinone (TQ) and its dimer dithymoquinone, thymohydroquinone (THQ), and thymol, which are known for their anticancer activity. The main phytochemical component of the volatile oil of *Nigella sativa* is TQ, which accounts for 28%-45% of the oil. TQ has been thoroughly studied *in vitro* and *in vivo*, and shown to have a range of therapeutic properties, including analgesic, antihypertensive, lipid-lowering,anti-inflammatory, antibacterial, antifungal, antihistaminic, antidiabeticand anticancer activity [9]. Inhibition of transcription of nuclear factor kappa B (NF-κB) has been suggested as a potential mechanism for the anticancer effects of TQ.A survey of literature revealed that abortifacient effects of *Nigella sativa*have not been documented in female albino rats. Hence, this study was undertaken to evaluate antioxidant and abortifacient effects of *Nigella sativa*in female albino rats.

2. MATERIAL AND METHODS

2.1. Plant material

The seed of plant *Nigella sativa* was collected in the month of November from the local market of Bhopal. Herbarium file of plant part was prepared and authenticated by Dr. Zia UlHasan (Professor), Safia College of science, peer gate, Bhopal (M.P.) and the specimen voucher no. assigned was 333/Bot/Safia/12. After that Herbarium file was submitted in Truba Institute of Pharmacy, Bhopal. Seeds was pulverized to coarse powder with the help of mixer grinder. The coarse powder was passed through sieve No22 to maintain uniformity and packed into airtight container and stored in cool and dry place. Thematerial was used for the further study.

2.2. Chemical reagents

All the chemicals used in this study were obtained from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade. Marketed preparation of mifepristone - Mifeprin 200mg brands of Sun pharmaceutical India ltd, B. No. JK92772, MFG. 05/2011, EXP. 04/2013 were purchased from Panchwati medicos, Airport Road Lal Ghati, Bhopal.

2.3. Extraction by soxhletion method

Powdered seeds (250g) of *Nigella sativa*was exhaustively extracted with different solvent (distilled water and ethanol) by soxhletion method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts.

2.4. Macroscopical evaluation

Macroscopical study is the morphological description of the seed which can be seen by naked eyes and it was performed by following the standard methods to determine the taste, size, color and odor of the seeds of *Nigella sativa* [10].

2.5. Physicochemical parameters

Physicochemical parameters suc as loss on drying, Total Ash value, acid insoluble ash value, water soluble ash value and foaming index were determined using standard procedures [11-12].

2.6. Phytochemical screening of the extract

The extract of *Nigella sativa*was subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids [13, 14].

2.7. Antioxidant activity

2.7.1. DPPH free radical scavenging assay

DPPH scavenging activity was measured by modified method [15]usingthe spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentrations (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume was made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally, the mean was calculated. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentrations after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. Though the activity is expressed as 50% inhibitory concentration (IC_{50}), IC_{50} was calculated based on the percentage of DPPH radicals scavenged. The lower the IC_{50} value, the higher is the antioxidant activity.

2.8. Animals

The experimental protocol was approved by Institutional Animal Ethics Committee. The rats were maintained under standard conditions in animal house approved by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA). The temperature was 23 ± 2°C and humidity was 50 ± 5%. Mature, healthy female albino rats weighing 180-200 gmwere used. They were provided with standard ratfeed (Amrut lab animal feed, Pranav Agro Industries Ltd., Sangli, Maharashtra) and water *ad libitum*.

2.9. Acute toxicity study

Oral acute toxicity study was evaluated as per OECD guidelines 423 (Organization for Economic Cooperation and Development) in female albino rats of Wistar strain [16]. Animals were provided by Truba Institute of Pharmacy, Bhopal and experiment was done in the Institute. Before experimentation, rats (n=3) were fasted overnight with water *ad libitum*. Animal were divided into five groups each consisting 3 animals. The first, second, third, fourth and fifth group received 200, 400, 800, 1200 and 2000 mg/kg body weight of *Nigella sativa* seeds extract (ethanolic and aqueous) respectively by gavage using intubation canula. Animals were observed individually after dosing any toxicity sign of gross changes like convulsion, tremor, circling, depression, and mortality. No significant signs were noticed in animals. Hence administered dose was found tolerable as no death was found. Therefore 400mg/kg body weight of ethanolic and aqueous extracts was selected for abortifacient activity.

2.10. Preparation of extract dose

A 2% acacia suspension was prepared by suspending 2 gram of accurately weighed acacia powder in 100 ml of 0.9% saline. 20 ml of vehicle was taken separately to which 4 gram of dried extract was added and sonicated, this produce suspension of 200 mg/ml strength. Both ethanolic and aqueous extract suspension was prepared in such manner.

2.11. Experimental designs (Abortifacient activity)

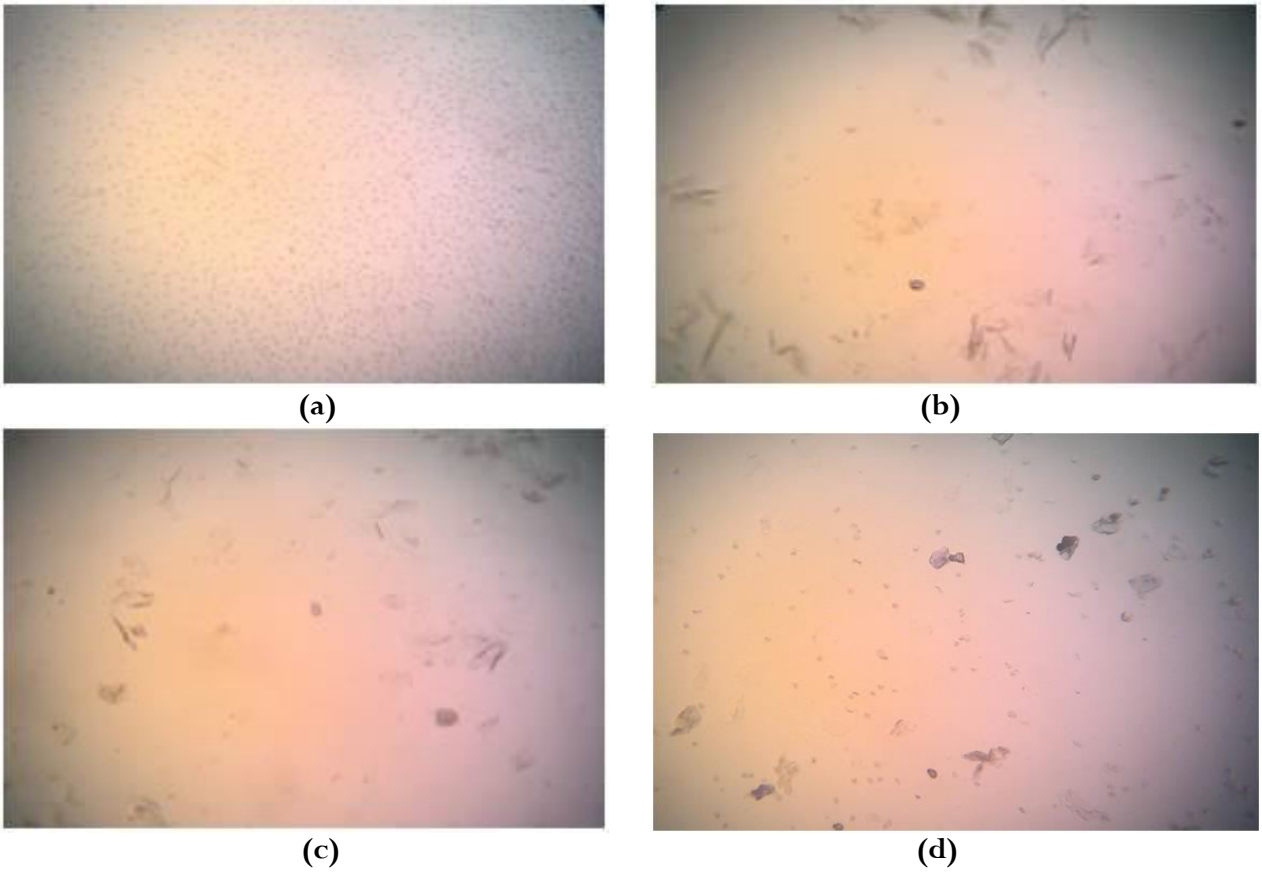
2.11.1. Pregnancy maintenance model

- Vaginal smear of each female Swiss albino rat was prepared daily between 10 and 10.30 AM continuously for 15 days. Female rats having normal estrus cycle were selected for the study of abortifacient activity.
- Vaginal smear was prepared by introducing a drop of distilled water into the vagina with the help of a dropper, collecting back and placing it on a clean slide after adding a drop of glycerin.
- Then prepared smear was examined microscopically under low power (10X, 40 X) for different types of cells (Fig.1). There are four phases in estrus cycle of rat. If majority of cells are leucocytes, then it was labeled as in diestrus phase (Fig. 1a). Presence of large number of nucleated cells indicated proestrus phase (Fig. 1b). Estrus phase was confirmed when the smear showed more than 50% cornified epithelial cells (Fig. 1c). Metestrus phase was indicated by the presence of many neutrophils and scattered squamous epithelial cells in the smear (Fig. 1d).

- The rats with three regular estrus cycles were divided into four groups of four each. Rats found in proestrus phase of cycle were caged with males of proven fertility, in the ratio 2:1 and examined the following morning for evidence of copulation. Rats exhibiting thick clumps of spermatozoa in their vaginal smears were separated and that day was designated as day 1 of pregnancy [17].
- Group I received vehicle only 1% Tween 80, p.o. and served as control. Group II received mifepristone 2.85 mg/kg p.o. and served as standard. Group III received aqueous extract at 400 mg/kg p.o. and group IV received ethanolic extract at 400 mg/kg p.o.respectively, from 8th to 20th day of pregnancy. On 21$_{st}$ day of pregnancy, all the rats were sacrificed under light ether anesthesia and both horns of the uterus were observed, afterwards, Number of live fetus in individual rats, Number of dead fetus in individual rats, Weight of fetus, No of rat aborted, No. of rats with vaginal bleeding and Percentage of abortion were recorded.

.% of abortion = (No. of rat aborted/No. of rat used) ×100

% of survival = [livefoetus/ (live foetus + dead foetus)] × 100



(a) Diestrus, (b) proestrus, (c) estrus and (d) metestrus.

Fig. 1: Vaginal smear of rat showing different phases of estrus cycle

3. RESULTS

The crude extracts so obtained after the soxhletionextraction process was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. The yield of *Nigella sativa* queous and ethanolicextracts was 5.6 and 5.9 % w/w respectively.

3.1. Pharmacognostical studies

Table 1: Morphological characteristic of *Nigella sativa* seeds

Characters	Observations
Colour	Black externally and white inside
Odour	Slightly aromatic
Taste	Bitter
Size	2-3.5 × 1-2 mm

3.2. Physiochemical analysis

3.3. Qualitative phytochemical analysis

3.4. Antioxidant activity

3.5. Pharmacological screening of abortifacient activity

Table 2: Physiochemical analysis of powder of *Nigella sativa* seeds

Parameters	Observations
Loss on drying	4 % w/w
Total Ash value	4.82 % w/w
Acid insoluble ash value	0.15 % w/w
Water soluble ash value	1.71 % w/w
Foaming index	24 (ml)

Table 3: Phytochemical screening of *Nigella sativa* seeds extracts

Tests	Ethanolic extract	Aqueous extract
Carbohydrates		
i) Molisch's Test	(+)	(+)
ii) Benedict's test	(+)	(+)
Tannins		
i) with 5%ferric chloride solution	(–)	(+)
ii)with 10% aqueous Potassium dichromate solution	(–)	(+)
iii) with 10% lead acetate solution	(–)	(+)
Alkaloids		
i) Dragendorff's Test	(+)	(+)
ii) Mayer's Test	(+)	(+)
Glycosides		
i) Legal Test	(+)	(+)
ii) Baljet Test	(+)	(+)
Flavonoids		
i) Shinoda's Test	(–)	(+)
ii) Alkaline reagent test	(–)	(+)
Steroids and Sterols		
i) Libermann-Burchard Test	(–)	(+)
ii) Salkowski Test	(–)	(+)
Proteins and Amino Acids		
i) Biuret Test	(+)	(+)
Saponins		
i) By shaking the extract in test tube	(–)	(+)

(+) = Present, (–) = Absent

Table 4: % Inhibition of ascorbic acid and extracts of *Nigella sativa* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition		
		Ascorbic acid	Ethanol extract	Aqueous extract
1	10	44.65	21.45	25.45
2	20	48.62	29.98	35.56
3	40	65.34	36.65	45.58
4	60	69.65	45.56	52.23
5	80	77.41	55.58	61.45
6	100	84.13	62.12	69.98
IC 50		17.68	70.13	54.37

Table 5: Abortifacient effects of the ethanolic and the aqueous extracts of *Nigella sativa* seeds in female albino rats

Parameters	Control	Standard (Mefepristone - 2.85 mg/kg)	Aqueous extract 400mg/kg	Ethanolic extracts 400mg/kg
No of live fetus in individual rat	10,11,9,9	0,0,0,0	0,0,0,4	3,3,0,0
No of dead fetuses	0,0,0,0	0,0,0,0	0,5,2,4	5,4,2,1
No of rat aborted	0	4	3	2
Weight of fetus (g)	1.28 ± 0.03	0.0 ± 0.0**	0.27 ± 0.27**	0.48 ± 0.27*
Percentage aborted	0	100	75	50
Survival ratio of Fetus (%)	100	0	26.66	33.33
No. of rats with vaginal bleeding	0	4	3	3

*Values are mean ± SEM. n=4 in each group. *P<0.05, *P<0.01when compared with control. Statistical significance between treated and control groups was analyzed using of ANOVA, followed by Dunnetts t-test.*

4. DISCUSSION

Morphological characteristics of *Nigella sativa* seeds are summarized in table 1. *It* is an erect annual herb up to 70 cm tall, with well developed yellow brown tap root and numerous feeder roots. The stem is profusely branched, ribbed, and sometimes hollow when old and light to dark green. Their seed colour has Black externally and white inside, slightly aromatic odour, bitter taste and having 2-3.5 × 1-2 mm. *Nigella sativa* seeds were shade dried and turned to powder for various physiochemical parameters like loss on drying, total ash value, acid insoluble ash value, water soluble ash value and foaming index which are summarized in table 2. Phytochemical investigation of various extract of *Nigella sativa*revealed the nature of phytochemicals present in their seeds which are summarized in the table 3 along with the chemical test applied for the different phytoconstituents. It was found that they contain abundant amount alkaloids, carbohydrates, glycosides, proteins and amino acids were found in both ethanolic and aqueous extracts. *Nigella sativa* were found to contain tannins in aqueous extract. Aqueous extract was also containing saponins, steroids and sterols. DPPH radical scavenging assay measured hydrogen donating nature of extracts [18]. Under DPPH radical scavenging activity the inhibitory concentration 50% (IC_{50}) value of *Nigella sativa*aqueous and ethanolextract was found to be 54.37 and 70.13µg/ml as compared to that of ascorbic acid (17.68µg/ml). A dose dependent activity with respect to concentration was observed (Table 4). Mortality and behavioral changes were not observed in the acute toxicity study till the dose of 2000 mg/kg. Therefore, doses 400 mg/kg were selected for the study. The feed and water intake were not significantly altered in all the groups. Mifepristone and ethanolic and aqueous extract produced varying effects on the parameters of abortifacient evaluated in this study. For example, the number of life foetus and the weight of the foetus decreased significantly (P<0.05, P<0.01). When compared with control. Statistical significance between treated and control groups was analyzed using of ANOVA, followed by Dunnetts t-test. In contrast, no life foetus was recorded in the animals treated with mifepristone as well as the 400 mg/kg, p.o. body weight of the ethanolic and aqueous extract having live foetus. Whereas ethanolic and aqueous extract having dead foetus and there was no foetus in the mifepristone treated animals. The survival rate of the foetus decreased from 100% in the control to 26.66% in the animals treated with 400 mg/kg, p.o. body weight of the aqueous extract and 33.33% of the ethanolic extract while none of the foetus survived in the mifepristone. There was 100% abortion in the animals treated with mifepristone as well as 75% abortion in the 400 mg/kg, p.o. body weight of the aqueous extract and 50% abortion in the 400 mg/kg, p.o. body weight of the ethanolic extract. The episodes of abortion in the animals were accompanied by vaginal bleeding (table 5 & Fig.2a-d).

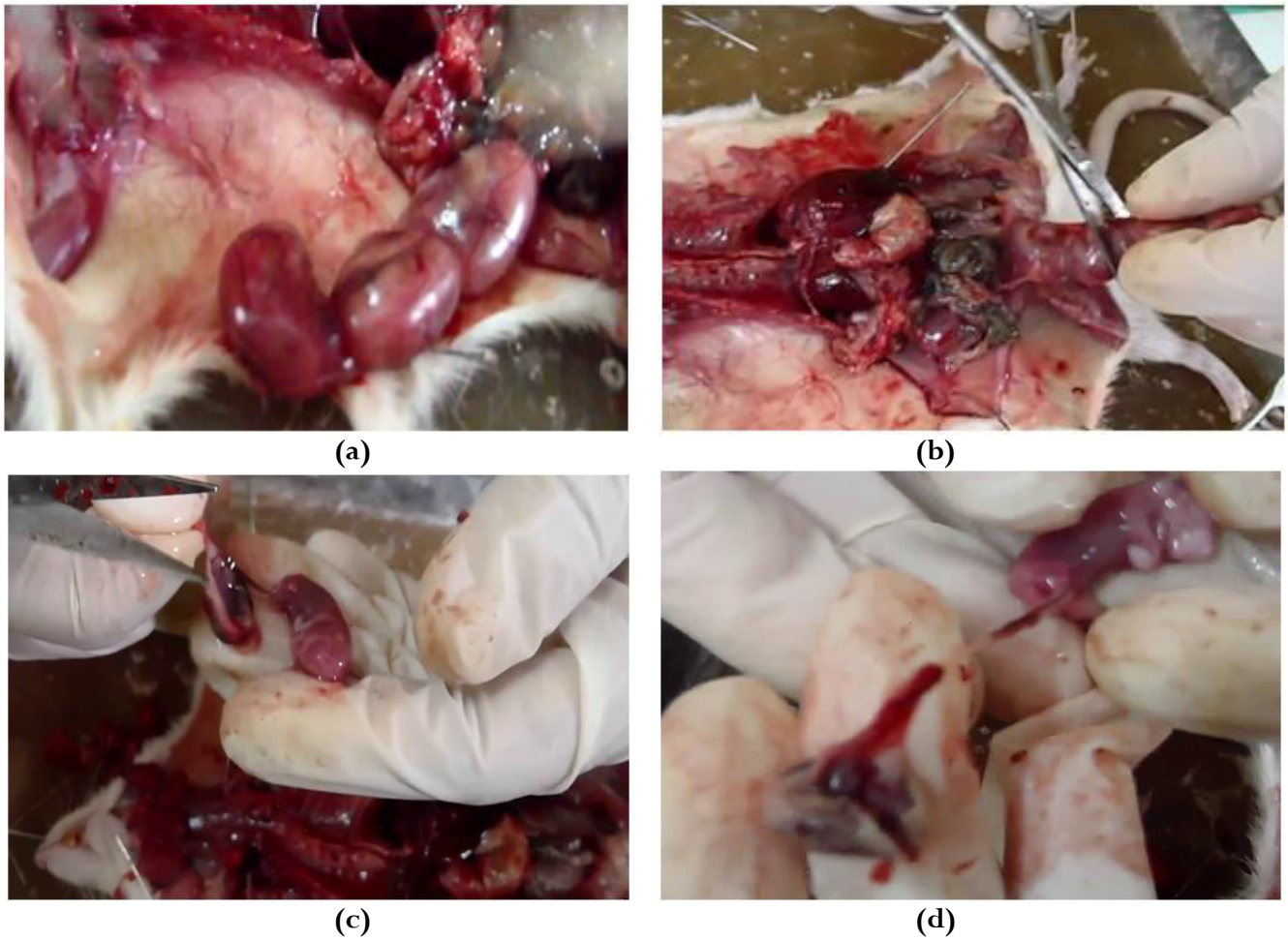


Fig. 2: (a) Pregnant female albino rat showing fetuses in uterus (b) Cutting of uterus (c) Removal of amniotic sac (d) Fetus attached to the placenta with Umbilical cord.

5. CONCLUSION

In this present investigation, the antioxidant ability of plant extracts were determined using DPPH free radical scavenging method, it can be concluded that seed of *Nigella sativa*have exhibited significant antioxidant activity with the presence of phytochemical constituents that leads to discovering new antioxidant agents in the pharmaceutical field. In this experiment, abortion is induced by using pregnancy maintenance model. In contrast, no live foetus was recorded in the animals treated with mifepristone as well as the 400 mg/kg, p.o. body weight of the ethanolic and aqueous extract having live foetus. Whereas ethanolic and aqueous extract having dead foetus and there was no dead foetus in the mifepristone treated animals.Finally,it was concluded that the active constituents of *Nigella sativa* ***seeds extract s***effective to induce abortion with no or very less complications, side effects or adverse effects. The survival rate of the foetus decreased from 100% in the control to 26.66% in the animals treated with 400 mg/kg, p.o. body weight of the aqueous extract and 33.33% of the ethanolic extract while none of the foetus survived in the mifepristone. There was 100% abortion in the animals treated with mifepristone as well as 75% abortion in the 400 mg/kg, p.o. body weight of the aqueous extract and 50% abortion in the 400 mg/kg, p.o. body weight of the ethanolic extract. The episodes of abortion in the animals were accompanied by vaginal bleeding. The actual mechanism of action of *Nigella sativa* extract against female albino rats is not clear with these studies.

Conflict of interest

None declared.

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