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Efficacy of Black Cumin (*Nigella sativa*) Ameliorate Organophosphate Toxicity in Pregnant Rats Based on Progesterone Levels and Fetal Bone Development

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Abstract

Black cumin (*Nigella sativa*), also referred to as black cumin, is a versatile herbal treatment that has recently grown in popularity, it contains antimicrobial, antibacterial, antifungal, anti-inflammatory, analgesic, antipyretic, antiparasitic, antihistamine, antihypertensive, antitumor, anticancer, antidiabetic, and anti-asthmatic properties are just a few of the positive and pharmacological effects of active ingredients in black cumin, such as thymoquinone and other compounds. This study aimed to evaluate black cumin activity ameliorates organophosphate toxicity in pregnant rats based on progesterone levels and fetal bone development. In this study, pregnant albino rats were divided into five treatment groups i.e. (K-) placebo; (K+) treated an organophosphate; treated an organophosphate and black cumin extract at a dose of (P1) 200 mg/kg BW, (P2) 400 mg/kg BW, and (P3) 600 mg/kg BW. The progesterone levels serum and fetal bone development were evaluated and calculated using ANOVA followed by Turkey. The results showed that P2 and P3 groups significantly increase in progesterone levels compared to K+ and P1 groups. Fetal bone was also reported increase in the growth of caudal vertebrae. In conclusion, 400 mg/kg BW of black cumin could ameliorated progesterone levels and fetal bone development in pregnant rats with organophosphate toxicity.

Keywords: black cumin, fetus, *Nigella sativa*, organophosphate, progesterone

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INTRODUCTION

Regularly applying large dosages of pesticides will result in several losses, including the accumulation of pesticide residues in agricultural products, pollution of the agricultural environment, lower production, poisoning of animals, and poisoning of humans with negative health impacts. Humans can pass away causing both acute and chronic poisoning (Khafaga *et al*., 2020). According to the World Health Organization (WHO), agricultural laborers suffer from 1–5 million pesticide poisoning events per year, with a mortality rate of 220,000 persons. Food, especially vegetables, is involved in 80% of agricultural poisoning. When applied frequently and in large doses, pesticides have the potential to be lethal. The World Health Organization (WHO) estimates that it is reported yearly in developing countries. Organophosphate pesticides can penetrate skin surfaces and enter the body by inhalation, digestion, and exposure or penetrating skin surfaces (Mu *et al*., 2019). Mahna and Sharma (2021) stated that the degree of poisoning is determined based on the blood's activity of the cholinesterase enzyme and is broken down into three categories: mild poisoning (50–75%), moderate poisoning (25–50%), and severe poisoning (0–25%).

Pesticide poisoning in pregnant women develops when pesticides enter the body through oral consumption, skin contact, and inhalation. Pesticides then impact fetal growth after crossing the placenta and getting into the fetus (Medvecky *et al*., 2015). Additionally, pesticides that enter the body run the risk of upsetting the hormonal balance of the female reproductive system (Prastiya *et al*., 2019). All parts of the hormonal system, including hormone synthesis, hormone release and storage, hormone distribution, and

hormone recognition and binding, can be impacted by these diseases, including the thyroid gland, the central nervous system, and all of the aforementioned (Hamid *et al*., 2019). This occurs as a result of pesticides' capacity to mimic, block, or otherwise interfere with the actions of bodily hormones (Khafaga *et al*., 2020).

Traditional medicine is advised as a way to keep one's health or prevent disease. The growth of traditional medicine and the back-to-nature movement have led to an increase in its popularity. Black cumin (*Nigella sativa*), is a multifunctional herbal treatment that has recently grown in popularity. Researchers are interested in demonstrating the pharmacological effects of the active ingredient thymoquinone and various other ingredients in black cumin, which has many advantages for humans, using a variety of preclinical and clinical trials in humans, experimental animals, and cell culture, as well as in vivo, in vitro, and molecular study. reducing sugars, alkaloids, organic acids, tannins, resins, yellow volatile oil (0.5–1.6%), mixed oil (35.6–41.6%), protein (22.7%), amino acids (albumin, globulin, lysine, leucine, isoleucine, valine, arginine, asparagine, cystine, glutamic acid, aspartic acid, proline, serine, threonine, and tyrosine) (Hosseinzadeh *et al*., 2013; Hamid *et al*., 2018).

The objective of this study was to use herbal remedies that are safe for both pregnant people and animals. One of these is black cumin, an herbal remedy with numerous applications whose use has recently grown increasingly widespread. The pharmacological effects of black cumin, which contains the active ingredient thymoquinone as well as several other components that have many beneficial effects on humans, are being investigated through a variety of preclinical and clinical trials in humans, experimental animals, and cell culture, as well as in vivo, in vitro, and molecular study (Hosseini *et al*., 2014).

This study aimed to investigate the effects of black cumin extract on progesterone levels and bone growth in the fetal bone development of pregnant rats with organophosphate toxicity.

MATERIALS AND METHODS

Ethical Approval

This study was approved under Certificate No. 081-KEP-UB-2020, the UB Research Ethics Commission has given its consent for the use of these test subjects.

Study Period and Location

Rats were reared in the Embryology Laboratory at Universitas Airlangga, Surabaya. This study performed from March–July 2020.

Animals

Before being utilized as study subjects, rats were fed pellets and given free access to water for a week. Rats were coded, weighed, and randomly distributed. Rats were used to generate five treatment groups i.e. (K-) placebo, (K+) treated with organophosphate, (P1) treated with organophosphate and black cumin extract at a dose of 200 mg/kg BW, (P2) treated with organophosphate and black cumin extract at a dose of 400 mg/kg BW, and (P3) was treated with organophosphate and black cumin extract at a dose of 600 mg/kg BW.

Pregnant mare serum gonadotropin (PMSG, Folligon®) and 10 IU human chorionic gonadotropin (HCG, Chorulon®) were both administered to rats. After 17 hours of monogamous mating with male rats, the vaginal plug was evaluated. The rats were gathered as the parent population of pregnant rats and were judged to be pregnant on day 0 if there is a positive vaginal plug. Additionally, randomization was used to choose the study sample. It is regarded as negative and is not used as a sample if there is no vaginal plug present. The clotted semen plasma and the vaginal plug are both components of the vaginal fluid (Nascimento *et al*., 2018).

Preparation of Black Cumin Extract

The dry black cumin was grind into powder using a blender. While stirring intermittently, black cumin powder was added to 70% ethanol and allowed to dissolve completely. Three solvent changes were made during the one-week

maceration process. The filtrate and residue are obtained during the filtration process. The filtrate, which has been filtered multiple times until it is clear, is then taken to the evaporation stage with a Rotatory Evaporator at a temperature of 40°C to produce black cumin extract, which is then dried with a freeze dryer (Muzolf *et al*., 2020).

Progesterone Evaluation

A total of 6 mL sample of blood serum from albino rats was analyzed for progesterone using an ELISA kit after undergoing various treatments. Then, an ELISA kit was used (E-OSEL-R0007) to determine the progesterone levels in the animals (Eltigani *et al*., 2021).

Alizarin Red Fetal Bone Staining Method

The Alizarin Red (catalog number: 0223, Scien Cell) method of fetal bone staining was used in this study. First, the fetus was gathered, and sacrifices were made (Isotupa and Virtanen, necropsy was taken a skin, and hair of the fetus were then fixed with a 96% ethanol solution for a week. After 24 hours of being submerged in a 2% KOH solution, the muscles will appear transparent. Then, it was poured into a 2% KOH solution that also included 0.005% Alizarin Red. Then, cleansed in a 2% KOH: glycerin solution after being soaked in 1% KOH for 24 hours in a 3:1 KOH: glycerin solution. Use KOH: glycerin 1:1 for 24 hours. To prevent the growth of fungi, items should be preserved with thymol and 100% glycerin (Kovacs, 2014).

Data Analysis

This study obtained quantitative data on progesterone levels and descriptive data on fetal bone growth. Quantitative data will be statistically evaluated with a 95% confidence level ($p > 0.05$) using one-way ANOVA analysis followed by the Tukey test (Benedetti *et al*., 2022).

RESULTS AND DISCUSSION

Progesterone Level

The examination of pregnant organophosphate-contaminated rats aims to evaluate how the progesterone levels in blood samples fluctuate expressed. The increase of progesterone hormone 10.10% in K- group was significantly different ($p < 0.05$) compared with P3 (Table 1), this value was related to the induction of organophosphates in pregnant rats' uteri, which may affect hormone secretion and lead to the production of reactive oxygen species (ROS). Increased ROS in the uterus of pregnant rats will cause fetal anomalies in development, inflammation, necrosis, rapid abortion of the fetus, and premature birth (Bahari *et al*., 2023). Black cumin extract does not yet have therapeutic activity; thus, there is still a change in progesterone secretion that impacts pregnancy in rats. P1 group revealed an increase in hormone levels compared to the K+ group. Hormone levels drop in P2 and P3 groups until they reach 17.38% and 11.44%, respectively (Table 1). This indicates that the dose of black cumin extract used in this therapy was therapeutic use of a dose of 400 mg/kgBW demonstrated the efficacious dose.

Table 1. Progesterone levels in all treatment groups

Groups	Mean	
	Increase in Negative Control (%)	Decrease in Positive Control (%)
K-	6.05 ± 0.62^{a}	0.00%
K+	6.73 ± 0.57^{ab}	10.10%
P1	8.02 ± 0.82^{b}	24.56%
P2	5.56 ± 0.26^{a}	17.38%
P3	5.96 ± 0.60^{a}	11.44%

Different superscripts in the same column indicate a significant difference ($p < 0.05$).

The luteal cells in the corpus luteum of the ovary secrete progesterone, a key hormone involved in reproduction (Saputro *et al*., 2020). The level of progesterone in the blood serum can indicate if an animal is infertile, normal, lustful, or pregnant. Progesterone affects both the development of secondary reproduction and the growth of the uterus during pregnancy. Pregnancy hormones like progesterone are among those whose secretion can be affected by the administration of organophosphates (Oyesola *et al*., 2019).

Black cumin contains flavonoids with anti-inflammatory, analgesic, and antipyretic properties, in this role showed as controlled hormonal levels demonstrate the anti-inflammatory impact, which is brought on by decreased uterine inflammation brought on by organophosphate-induced ROS (Rahma *et al*., 2017). According to this study, the active ingredient in black cumin is linoleic acid, which has an estrogenic activity that progressively raises the blood's level of estrogen and thickens the vaginal epithelial cells. Through this method, neurons release lumped parameter thermal network (LPTN), GnRH, and nitric oxide, in addition to having an immediate impact on the hormone (Sucita *et al*., 2019). Nitric oxide production in the hypothalamus is similarly increased by LPTN, while pituitary hormone levels (LH and FSH) are decreased. Antioxidants can promote oogenesis because the active ingredients in black cumin can obtain free radicals. Several black cumin constituents, such as flavonoids, anthocyanins, carotenes, isothiocyanates, and carotenoids, work in conjunction with antioxidants to eliminate ROS (Triana *et al*., 2020). Because black cumin oil contains UFAs such as linoleic acid (about 60%) and oleic acid (about 20%), it can raise progesterone levels and destroy defective ova. The presence of phenolic compounds and alkaloids may lead to a rise in the levels of estrogen and FSH in testicular tissue (Albores *et al*., 2021).

Fetal Bone Development

Alizarin red can be used to visually observe the composition of the spinal structure. By comparing the vertebrae of two sets of K- group and treatment groups with black cumin extract were able to create the figure.

In Figure 1 showed the progressive growth of bones in the fetus. Compared to the red arrows, caudal vertebrae mature more slowly and with a different size pattern. By contrasting the caudal vertebrae's growth patterns in the treated and control, it is possible to inspect the growth in the fetal spine. In the K+ treatment, the caudal vertebrae's growth pattern suffered a delay in ossification (short bone size) and the presence of flexion, in contrast to the caudal vertebrae's typical pattern of ossification and size. In P1 group, there was a delay in the ossification and flexion of the caudal vertebrae bone. Induced organophosphate caused delays in the ossification and flexion of the P2 caudal vertebrae bone. In P3 group, caudal vertebrae grew normally sized and exhibited mild flexibility throughout therapy.

The fetus with abnormal ossification was smaller and less uniform in size than the normal fetus, which had seven cervical bones, thirteen thoracic bones, six lumbar bones, six sacral bones, and two to three caudal bones (Li *et al*., 2019). Visual or descriptive observations of the anatomy of the spinal column are both possible. By contrasting the fetal spines of all groups, including the K- group and black cumin extract group treatments, it is possible to determine the findings of the study observations (Figure 1).

Fetal body length is a sensitive metric for detecting growth constraints in the fetus. Visual examination contrasting the spines of fetuses from mice induced by organophosphate 40 and black cumin extract at a dosage of 200 mg/kg BW, 400 mg/kg BW, and 600 mg/kg BW at cervical bones, thoracic bones, lumbar bones, sacral bones, and caudal bones make up a typical fetus (Li *et al*., 2019). According to the findings of this study, there were variations between the control and treated group of fetuses in terms of the number and structure of vertebrae as well as the outcomes of the Alizarin Red staining, as it showed changes in the number of vertebrae in the fetus for seven days.

The teratogenic expression of black cumin extract's active ingredients has the mildest effect on fetal body length, which causes growth inhibition in this case due to disruptions in fundamental bodily functions like metabolism, cell synthesis, and cell division (Tomaszewki *et al*., 2016). The fetus will grow and develop during gestation, both in terms of size and the development of fetal organs. The placenta serves as the fetus's conduit for nutrition from the mother during its time of growth and development. Through the placenta, xenobiotic chemicals that

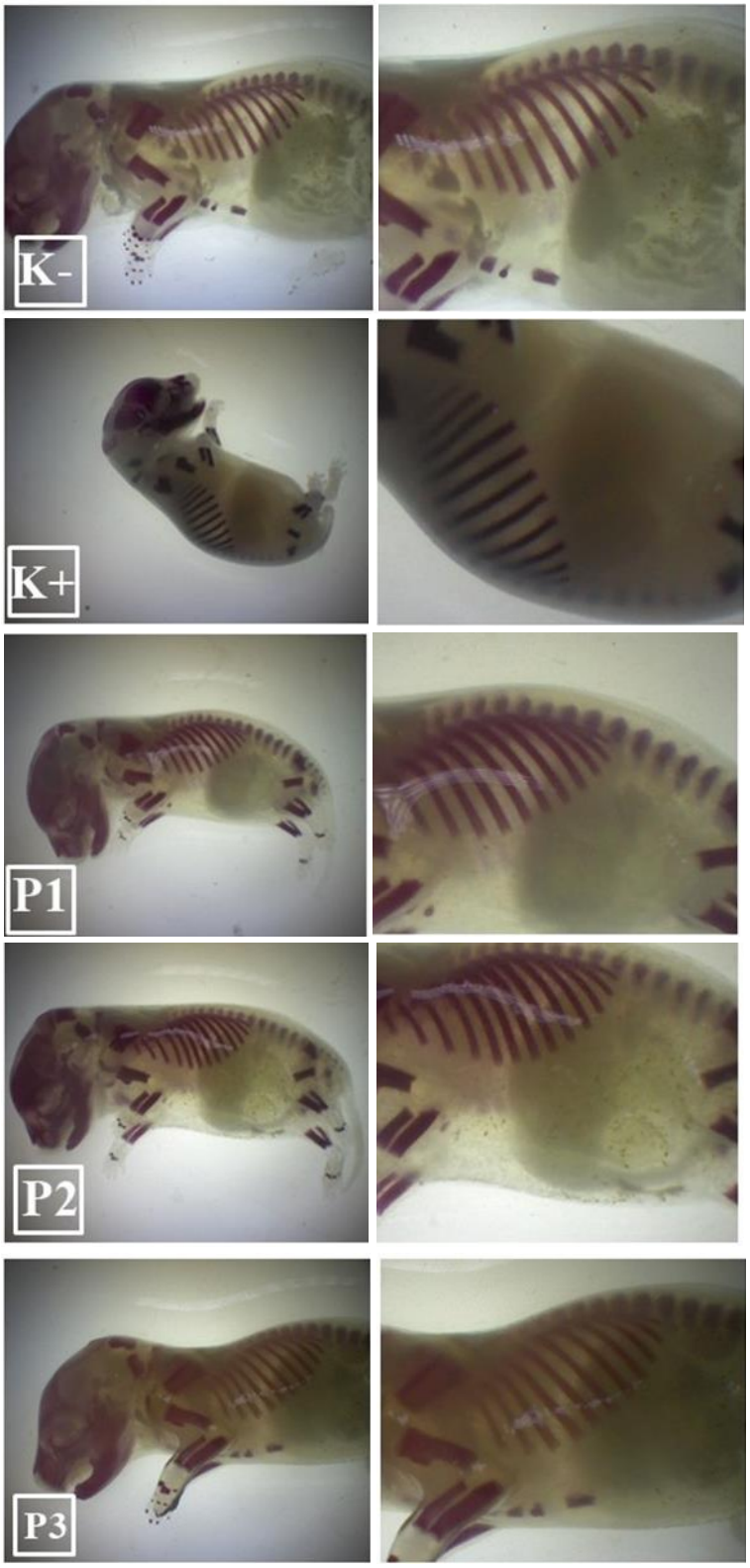

Figure 1. Alizarin red staining in caudal vertebrae of the fetus.

circulate in the parent's blood vessels will enter the fetus's body (Brett *et al*., 2014).

Organophosphates can disrupt hormone receptors, including those for thyroid hormone, which will affect the fetus's health. The fetus is affected by the hypothyroidism brought on by this. Thyroxine (T4) and triiodothyronine (T3) make up thyroid hormones. These hormones maintain the metabolism of different tissues, body growth processes, brain development, nervous

system development, and dental and bone tissue systems (Purnama *et al*., 2019; Kartikasari *et al*., 2020). One of the criteria that is frequently checked to determine whether a drug has teratogenic potential is skeletal growth. The existence or lack of ossification, the number and degree of ossification, and structural flaws like wavy ribs, extra ribs, or extra fingers are all things that can be observed when studying the development of the skeleton (Hidayatik *et al*., 2021). A fetus will grow and develop during gestation, both in terms of size and the development of fetal organs. The placenta serves as the fetus's conduit for nutrition from the mother during its time of growth and development. Through the placenta, xenobiotic chemicals that circulate in the parent's blood vessels will enter the fetus's body (Aroniadou *et al*., 2020). According to Chen *et al*., 2019, the number and degree of ossification, the presence or absence of ossification, and the presence or absence of structural flaws such as the existence of wavy ribs, extra ribs, or fingers are all examples of skeleton development observations.

Water, protein, fat, calcium, vitamin A, vitamin B2, ascorbic acid, niacin, fiber, and ash are some of the chemical components found in black cumin (Nadi *et al*., 2020). Black cumin contains the aforementioned chemical components in addition to essential extracts: 15 amino acids (alanine, arginine, isoleucine, lysine, tryptophan, tyrosine, asparagine, cystine, glycine, glutamic acid, methionine, and proline), iron, sodium, potassium, thiamine, riboflavin, pyridoxine, niacin, copper, and zinc. Thymoquinone (TQ), dithymoquinone (DTQ), thymohydroquinone (THQ), and thymol (THY) are the primary active components of black cumin. (Fikri and Purnama, 2020) In addition, nigellone and glutathione in black cumin serve as defense mechanisms for the body against numerous xenobiotic (Sikary, 2019). The active ingredients in black cumin extract are assumed to be able to stop the cell cycle, operate as an anti-proliferative, and induce apoptosis, which is thought to be the cause of the growth disturbance (Shoeib *et al*., 2003; Sukmanadi *et al*., 2021). Fetal bodily fluids probably include black cumin extract, which prevents cell division. Black cumin extract contains active ingredients like essential oils, alkaloids, and flavonoids that are responsible for this inhibitory effect (Sukmanadi *et al*., 2021). This is possible because the fetal body can still tolerate the black cumin extract due to the presence of nutrients and minerals as well as the fetus's capacity to recover (Dagg *et al*., 2014).

CONCLUSION

In conclusion, an administration of 400 mg/kg BW of black cumin extract could increase progesterone levels. In addition, based on alizarin red interpretation of fetal bone didn't show the occurrence of flexors, and the size of the fetal bones appeared normal but affected the pattern of ossification of the caudal vertebrae.

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AUTHORS' CONTRIBUTIONS

VFH: Conceptualization and drafted the manuscript. YO, AF, and VFH: Validation, supervision, and formal analysis. GCA, SA, YO and DM: Performed sample evaluation. AF and VFH: Performed the statistical analysis and the preparation of table and figure. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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