

# **The Effect of Aqueous Extract of Poppy Anemone Seeds on Embryonic Development in Mouse Model**

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## **Abstract**

The current study aims to investigate the tissue lesions induced by Poppy anemone in pregnant mice. The administration of Poppy anemone (200,400 and 600 mg/kg) begins from days 7-8 of pregnancy. The results showed an increase in the rate of deaths and miscarriages in pregnant female mice with an increase in the concentration used. The uterine horns also showed a difference in the distribution of embryos, the appearance of bleeding cases and the accumulation of fatty materials. Some absorbed embryos also appeared. As for histological changes in the skin of the fetuses, there was edema around the hair follicles and between the collagenous fibers of the dermis layer and around the skin, sweat, and sebaceous glands, presence of edema between the epidermis layer and the dermis, congestion of capillaries, the divergence of collagen fibers in the dermis of the skin, hyperkeratosis of the epidermis, presence of hydrophilic degeneration in the spiny cell layer of the epidermis, rarefaction of the white fibers of the dermis and congestion of blood capillaries.

**Keywords:** Poppy anemone, Histological changes, Pregnant mice.

## تأثير المستخلص المائي لبذور شقائق النعمان على التطور الجنيني في نموذج الفأر

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### الخلاصة

اجريت الدراسة الحالية لفحص الآفات النسيجية المحدثة بواسطة شقائق النعمان الخشخاش في الفئران الحوامل. يبدأ تناول شقائق النعمان الخشخاش (200.400 و 600 ملغم/كغم) من اليوم السابع إلى اليوم الثامن عشر من الحمل. وأظهرت النتائج، أظهرت النتائج زيادة في معدل الوفيات والإجهاض لدى الأمهات الحوامل مع زيادة التركيز المستخدم. كما أظهرت قرون الرحم اختلافاً في توزيع الأجنة وظهور حالات النزيف وتراكم المواد الدهنية. كما ظهرت بعض الأجنة الممتصة. أما التغيرات النسيجية في جلد الأجنة فتظهر الوذمة حول بصيلات الشعر وبين الألياف الكولاجينية لطبقة الأدمة وحول الجلد. الغدد العرقية والدهنية وكذلك وجود وذمة بين طبقة البشرة والأدمة، احتقان الشعيرات الدموية، تباعد ألياف الكولاجين في أدمة الجلد، فرط تقرن البشرة، وجود تنكس محب للماء في طبقة الخلايا الشوكية البشرة، وتخلخل الألياف البيضاء في الأدمة، واحتقان الشعيرات الدموية

الكلمات المفتاحية: شقائق النعمان الخشخاش، التغيرات النسيجية، الفئران الحوامل

### Introduction

*Papaver rhoeas* - L, also known as field poppy, Corn Rose, Shirley poppy, or Flanders poppy, is an annual plant of the poppy family *Papaveraceae* 30-60 cm in height. Members of this family are distributed mainly in North Temperate zones. *Papaver rhoeas* is widely grown as a weed in cereal naturalized in country roads and fields worldwide (Grauso *et al* 2021). This plant is large and showy, with four red petals with a black blotch at the central base. Phytochemical and physiological characterization of *Papaver rhoeas* belongs to the opium alkaloids, closely related to benzyloisoquinoline and contains various powerful alkaloids such as rhoeadine alkaloids (Günaydın *et al* 2015, Çoban *et al* 2017, Oh *et al* 2018). This makes *Papaver rhoeas* plant narcotic and effective sedative relief of such pains (Zargari, 1994, Oh *et al*. 2018, Grauso *et al* 2021). The plant, therefore, serves as opium due to the same qualities, but it does not contain morphine nor does it stimulate addiction (Kültür 2007). Poppy is also rich in linoleic acid at 74.5% (Bozan and Temelli, 2008).

*Papaver rhoeas* are used as food among the local people (Günaydın *et al* 2015). Seeds and leaves are probably the most widely cooked and eaten part, although all of *Papaver* parts probably are toxic due to their high conceivable intoxication by alkaloids (Günaydın *et al* 2015, Çoban *et al* 2017, Oh *et al* 2018). Petals are also collected to make a red colouring or as moisturising

treatments for itchy and redness in the skin (Mitich, 2000, Mabberley, 2017). *P. rhoeas* antimicrobial activity has been tested (Çoban *et al* 2017) and thus used as a drug (Rahfeld, 2017), and plays an important role in medicinal use. It is used to treat cough or sleep disorder and is useful in various states such as pneumonia, rash fever, and bronchitis (Pourmotabbed *et al* 2004). Moreover, it is also established in ethnopharmacology that *P. rhoeas* can be used to treat insomnia, nervousness, respiratory, and digestive disorders, eye infection, and baldness as well as for treatment of measles (Morales *et al* 2014). However, *P. rhoeas* is also known to cause health effects in humans such as urinary irritation and intestinal, pneumonia, arrhythmia, nausea, seizures, vomiting, myosis, bronchitis, confusion, and rash fever (Rey-Caballero *et al* 2015, Osanloo *et al* 2016, Günaydın *et al* 2015, Koçak *et al* 2016, Zhou *et al* 2018). This present research, therefore, was undertaken to evaluate the possible effects of *Papaver rhoeas* extract on the skin of embryo using mouse as a model for the study.

## Materials and Methods

The current study was conducted on pregnant female Swiss albino mice (*Mus musculus*) aged 9-12 weeks weighing  $23 \pm 2$  grams, and in good health. The mice were kept under uniform laboratory conditions with controlled ventilation and temperature ( $24 \pm 2$  °C), and a 12-hour light-dark photoperiod (Al-Mamouri, 2001). The mature females were mated with males (1 male / 3 females), and successful mating was confirmed by the presence of a vaginal plug. The day of mating was considered day zero of pregnancy, and the following day was considered the first day of pregnancy (Biernacki *et al.*, 2000).

An aqueous extract of the plant Poppy anemone was used in the experiments. Twenty pregnant female mice were divided into four equal groups, including a control group given only distilled water, and three experimental groups administered extract once daily from the seventh day of pregnancy until the eighteenth day of pregnancy, as the following schedule: -

| The number mice | duration of administration | Concentration milligrams per kilogram | groups           | The period  |
|-----------------|----------------------------|---------------------------------------|------------------|---|
| 5               | 11                         | Distilled water                       | control(1)       | Oral from the seventh to the eighteenth day of pregnancy. |
| 5               | 11                         | 200                                   | Experimental (2) |   |
| 5               | 11                         | 400                                   | Experimental (3) |   |
| 5               | 11                         | 600                                   | Experimental (4) |   |

The pregnant female mice were orally administered with the aqueous extract of the Poppy anemone plant using a gavage needle. The pregnant females were examined on the eighteenth day of pregnancy, and a visual inspection of the fetuses was conducted. The fetuses were fixed in Bouin's solution, and tissue sections were prepared according to the method described by Luna (1968). The fixed samples were washed with 70% ethyl alcohol, dehydrated using ascending concentrations of ethyl alcohol, and then embedded in pure paraffin with a melting point of 54°C, poured into iron molds, and cut into 7-micron thick sections. These sections were stained with hematoxylin-eosin and Masson's trichrome stain to enhance the results (Bancroft and Steven, 1982). The slides were mounted with DPX mountant examined under a compound light microscope and imaged using a compound microscope equipped with a digital camera (Olympus OM-Japan).

## Results and Discussion

### Abortion and Uterine Horns

The results indicated that the abortion rate in the experimental group administered with the extract at a concentration of 400 mg/kg body weight was 15%, with a 13% incidence of maternal deaths. At a concentration of 600 mg/kg body weight, the abortion rate was 40%, with a 22% incidence of maternal deaths on the sixteenth and seventeenth days of gestation. These results are consistent with scientific literature, which suggests that high concentrations of *Colchicum autumnale* leads to the increase of spontaneous abortion and foetal deformities (Al-Mustafa and Al-Sultan, 2022). As for the deaths of some pregnant mice, it can be attributed to certain potent compounds present in the plant, such as protoanemonin, which can cross the placental barrier, reaching the foetal blood and causing harm, such as premature birth, respiratory problems, and increased risk of infection (Najjar *et al* 2022). As for the uterine horns, the results revealed some abnormalities in the experimental group administered with a concentration of 400 mg/kg body weight, manifested by irregular foetal distribution and severe congestion (Figure 1). At a concentration of 600 mg/kg body weight, an increase in cases of bleeding and blood congestion within the uterine horns was observed, along with partial accumulation of adipose material between the horns and the appearance of absorbed fetuses, indicating partial miscarriage (Figure 2). This is consistent with the findings of (Al-Barwari, 2013) regarding partial miscarriage, the appearance of absorbed fetuses in one uterine horn, dense accumulation of adipose material, and increased blood bleeding within the horns when rats were injected with the drug Clavaran at a concentration of 5000 mg/kg body weight. These effects demonstrate the harmful side effects of the substances used, especially at high doses. These results are similar to the findings of Tayfur (2009) regarding the appearance of absorbed fetuses and uneven foetal distribution within the uterine horns when pregnant rats were administered the drug Dexamethasone.

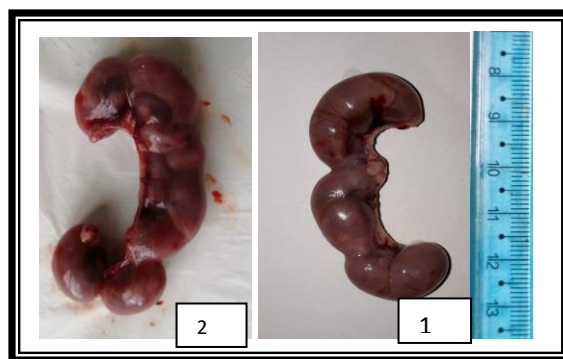


Figure 1: Uterine horns of pregnant female Mice administered with the aqueous extract of Poppy anemone at a concentration of 400 mg/kg body weight, showing severe congestion (1).

Figure 2: Uterine horns of pregnant female mice administered with the aque extract of Poppy anemone at a concentration of 600 mg/kg body weight, showing blood bleeding (1), blood congestion (2), partial accumulation of adipose material (3) between the horns.

### Morphological Description of Foetal Deformities Induced by the Aqueous Extract of Poppy anemone

Results of the present study demonstrated that when administered at a concentration of 400 mg/kg body weight during pregnancy, the percentage of deformed fetuses was 5%, with an average weight of 1.43 g and an average length of 21.22 millimeters, compared to the control group with an average weight of 1.02 grams and an average length of 20.71 millimeters, respectively (Figure3).

Figure 3: Side view of an 18-day-old white mouse foetus

from the control group injected with distilled water.



The deformities observed included head deformities in 15% of the fetuses, characterized by a downward curvature of the skull due to the appearance of a protrusion in the cranial vault and facial feature enlargement (Figures 4 and 5). These results are similar to those reported by (Al-Barwari ,2013) in pregnant rats injected with Clavoran at a concentration of 2000 mg/kg body weight, resulting in enlarged and elongated fetuses, possibly due to the accumulation of body

fluids, which is similar to what was mentioned by Ormerod (2001) or an increase in the volume of body fluids (Jauniaux and Burton, 2007). The results also indicated eye deformities and bleeding around them, similar to what was reported by Al-Naimi (2012) when pregnant female rats were administered with a black tea aqueous solution at a concentration of 2000 mg/kg body weight, resulting in congestion around the eyes and changes in their positions, which is similar to what was.

**Figure 4:** shows a lateral view of a mouse fetus from a mother that was administered a concentration of 400 mg/kg body weight, which illustrates the fetus's curvature and its rounded appearance (arrow), the head's protrusion and downward bending due to the skull's curvature, bleeding around the eyes (2), and ear ossicle deformity (3) and brain herniation (4).

**Figure 5:** A lateral view of a mouse fetus from a mother that was administered a concentration of 600 mg/kg body weight, shows the appearance of a hemorrhagic tumor above the skull, known as encephalomeningocele (1), eye deformities (2), and ossicle deformity of the ear (3).

Researchers indicate that ketamine anesthesia increases programmed cell death in neurons, and this effect varies with the dosage, duration of administration, and maternal age (Stratmann (2011) and Brambrink *et al.* 2012). It may disrupt embryonic development and lead to deformities. This is supported by the findings of Medion *et al.* (2007), who suggested that the trunk deformities observed, such as severe curvature and swelling, align with the impact of administering the drug pyrazinamide at concentrations of 2500 and 3000 mg/kg body weight to pregnant rats, as reported by Al-Sultan (2005). Furthermore, the observed swelling in the abdominal region may result from excessive drug dosage and its metabolic byproducts, which can affect embryonic development and the synthesis of essential proteins (McKee *et al.*, 2006), hindering the growth of the fetal skeletal system during pregnancy (Al-Enazy & Al-Mutairi, 2007).

Additionally, the appearance of brain herniation in the cranial region and spinal curvature from the posterior region towards the head aligns with the findings of Rebecca *et al.* (2009), who suggested that caffeine could cause the appearance of cranial herniation due to the failure of neural tube closure and the occurrence of spinal deformities, resulting in spinal cord swelling on the

dorsal surface covered by the skin (Abdel Majeed, 1999). Alternatively, the high dosage of anesthetic agents and the inability of maternal and fetal tissues to eliminate the toxicity of these substances may be the cause of cran region deformities (Edler *et al.*, 2012).

Due to the high doses used in the current study, the failure of the anterior part of the neural tube to close and the resulting deformities in the brain, as well as the appearance of tumors in the cranial region all were caused by tissue changes in the cellular organs that disrupted the process of fetal development, resulting in visible deformities such as trunk and abdominal deformities (Gao and Yang, 1996), similar to what was observed in the current study. Limb deformities were also observed in 18% of the fetuses, characterized by thickening and elongation of the anterior limbs and the separation of their fingers, as well as thickening and fusion of the posterior limbs' fingers. Tail deformities were observed in 18% of the fetuses, characterized by the appearance of a twisted or thin, short, tapered tail (Figure 4).

When the concentration was increased to 600 mg/kg body weight, the results showed that 68% of the foetuses were deformed, with an average weight of 0.95 g and an average length of 16.80 mm compared to the control group's respective averages of 0.96 g and 18.04 mm. The deformities observed included blue-skinned and deformed fetuses, with a 35% incidence of head deformities characterized by the curvature and rounding of the head and the flattening of the skull's curvature, as well as the unclear facial features, and the appearance of a hemorrhagic tumor above the skull, known as encephalomeningocele.

These results are consistent with what Al-Naimi (2012) found in the posterior brain region when administering Coca-Cola syrup at a concentration of 4 mg/kg body weight. This may be due to a defect in the growth of the middle part of the neural tube, causing the brain to protrude through a hole in the skull, or due to the ability of the anesthetic or analgesic substances, or their metabolites, to cross the blood-brain barrier (Grond and Sablotzki, 2004). The results are also agreed with what (Hamoudi,2005) reported on head and ear ossicle enlargement when administering pregnant mice with paracetamol at a concentration of 50 mg/kg body weight. A curved, downward-pointing head with a beak-like end was also observed. These results match what (Al Fathi ,2005) indicated, which is the enlargement of the head, resembling a bird's head, when administering pregnant mice with deltamethrin at a concentration of 300 mg/kg body weight, and eye deformities with a 48% incidence. These results, represented by eye bulging, agree with what Cook (2003) reported on the appearance of eye bulging when using alcohol during pregnancy, and with what Al-Sultan (2005) reported on the eye bulging of mouse fetuses when administering their mothers with rifampicin at a concentration of 250 mg/kg body weight. They also agree with what (Al-Hayali,2004) reported on the protrusion of the eyes in pregnant mice when consuming high doses of vitamin, A, at a dose of (IU10000) in the early stages of pregnancy. This may be due to the high dose of the substances consumed, which are harmful to the eyes, and optic neuritis has been recorded when using high doses of Ethambutol.



(Citron and Thomas, 1986) have reported a 52% deformities in the trunk, characterized by the distortion of the cervical flexure and the straightening of the dorsal trunk region, as well as the swelling and congestion of the abdominal dorsal region. Additionally, they observed skin deformities, including wrinkling and dermatocoele, indicative of skeletal system deformities. These findings are in line with the conclusions of (Najjar *et al* 2022), who explained that skin wrinkling and skeletal system deformities in pregnant mice may be attributed to the negative effects of smoke exposure. The reason of that could be related to the impact of drugs or smoke toxins on the development and differentiation of the integumentary system, leading to skin deformities during fetal development in mice. These results are also consistent with the findings of Oyedele and Kramer (2008), who suggested that alcohol administration to mice may lead to genetic mutations, disrupting the cellular mechanisms and causing damage to foetal tissues and organs, particularly the integumentary system (Streffer and Müller, 1996). Additionally, the appearance of deformities

In addition to congestion in the abdominal region, indicative of hemorrhagic bleeding, these results are consistent with the findings of Fujmora *et al.* (1965), who observed abdominal cavity fissures in rat neonates when using Captopril at a concentration of 200 mg/kg. Limb deformities, representing 24% of cases, included shortening and swelling of the forelimbs, as well as curvature of the hindlimbs, aligning with the observations of Miller and Hyatt (1992) regarding the diverse limb deformities caused by narcotic drugs. The cause of finger separation can be attributed to variable interactions between the superficial and intermediate dermis, which give rise to the cartilages forming the limb bones, and the occurrence of cellular death in the superficial dermis due to the cytotoxic effects of cellular factors (Scott *et al.*, 1975). These findings corroborate the observations of Hunter and Warren (1997) that the administration of Bayerazinamide at a concentration of 2500 mg/kg body weight causes shortening and deformities in both forelimbs and hindlimbs. These results are also consistent with the findings of Blekley and Scott (1984), who forelimb shortening and deformities in pregnant mice administered with ethyl alcohol at a concentration of 25%. The study revealed swelling and hemorrhage in the forelimbs, similar to the findings of Dehkordi *et al.* (2014) regarding the inflammatory and hypertrophic effects of high doses of Tramadol, as well as the synovial membrane. Additionally, the study recorded the enlargement and curvature of the forelimbs and hindlimbs, similar to the observations of Naimi (2012) regarding the effects of high doses of caffeine-containing beverages on pregnant mouse fetuses, causing curvature, atrophy of some fingers, and adhesion of others. The separation of the fingers at the centre may be attributed to the absence of programmed cell death between the membranes of the fingers, resulting in the adhesion of the fingers in those fetuses (Hurle and Granan, 1986). The cause may also be attributed to the lack of cell division in the intermediate epidermal cells between the hand or footplates, leading to the adhesion of some fingers or the fusion of bones (Al-Hamoud, and Youssef 2005). Perhaps it is due to the deviation of cellular interactions in the limb-forming regions, as well as cellular death in multiple areas of the limb, changes in the behavior of the intermediate epidermis, inhibition of cartilage formation and



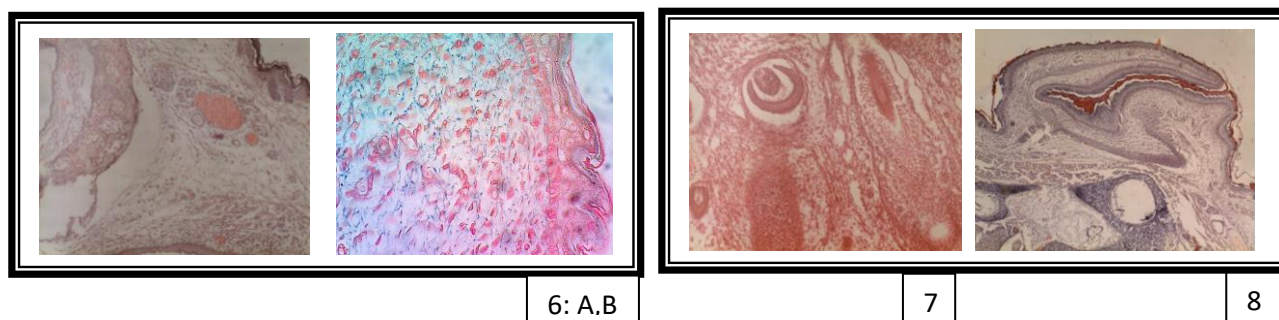
deformation, thus deforming the limbs and fingers (Kochhar, 1977). This explanation aligns with the findings of Wang *et al.* (2005) that treatment with ketamine increases the programmed cell death pathway. As for the tail, the deformity rate reached 30%, represented by the appearance of a thick hooked tail and a thin, twisted, tapered, spiraled tail end.

**: the pathological tissue changes in the covering system of the rat embryo's with the aqueous extract of the Colchicum plant.**

In many places in the world, *P. rhoeas* flowers color the landscape with their characteristic red petals, and historically the large availability of such flowers met human needs, with *P. rhoeas* being used in phytotherapy and also as food (Çoban *et al* 2017) *P. rhoeas* is used in the treatment of digestive system, nervousness, respiratory, eye infections, baldness and measles (Grauso *et al* 2021). It can be also cooked as its leaves are edible. In addition, poppy seeds are considered the most commonly eaten part, which is often rich in oil (47%) and about 21% protein (Earle and Jones 1962). Compounds of *P. rhoeas* ovule were probably open perspectives for further studies on cell lines and human cancer research. Despite the increase in the literature about *Papaver rhoeas* (Osanloo *et al* 2016, Günaydin *et al* 2015, Oh *et al* 2018, Ekici 2014, Sangy *et al* 2020, Zabalza *et al* 2020, Yang *et al* 2021, Katarzyna *et al* 2021), little data are available about *Papaver rhoeas* cutaneous toxicity. The present study, therefore, examined the toxic effect of *Papaver rhoeas* L. PRR (red flower PR) on the skin of mouse embryos. Three concentrations of *Papaver rhoeas* seeds extract were tested on the pregnant mouse. The current study demonstrated that this plant has a potential impact on the skin. Although some plants including poppy can be used in the treatment of reddened skin (Mitich 2000; Mabberley 2017). which can also be extracted, the current results demonstrated a harmful potential after cutaneous exposure to *Papaver rhoeas*.q

The microscopic examination results of the embryos in mothers treated with Poppy anemone indicated tissue changes in the embryos' skin, characterized by the appearance of edema around the hair follicles, between the collagen fibres of the dermal layer as well as around the sweat and sebaceous glands. The results also showed congestion of the blood vessels, as well as a separation of the collagen fibres in the skin dermis. (figure 7). Additionally, the results revealed the appearance of vesicular degeneration in the stratum spinosum of the skin, with the presence of edema in the deeper layers of the dermis and slight infiltration of leukocytes. It is expected that this area is the fetal Arabian area, as congestion and slight bleeding in the urinary bladder are observed, along with incomplete ossification in the pelvic bones, which still resemble cartilage Neutrophil granulocytes are prominent in many chronic inflammatory skin disorders (Schön, 2000) (figure 8). Furthermore, the excessive keratinization of the skin's horny layer and the presence of swelling (vesicular degeneration) in the stratum spinosum of the skin, along with loosening of the dermal collagen fibers and congestion of the blood capillaries were evident (Jacob *et al* 2023) Giving some substances to rats may cause tissue damage to the skin. (figure 9) This

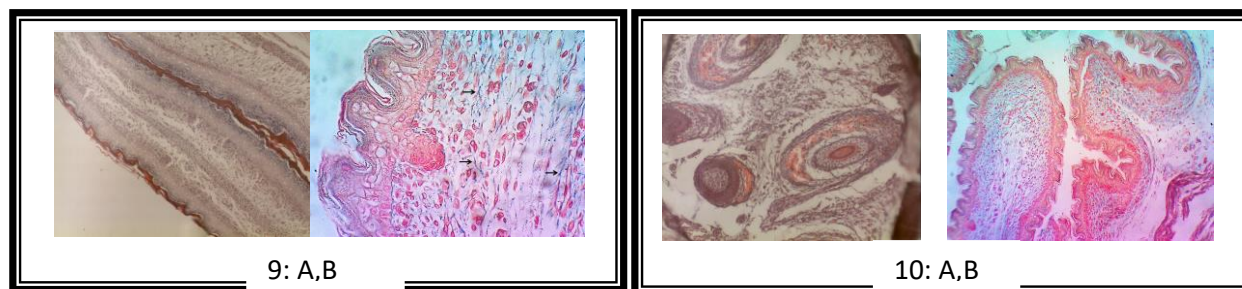
may be due to intercellular adhesion molecule-1 (ICAM-1) expressing activated epidermal keratinocytes](Clark and Kamen 1987) Additionally, there was congestion of the blood capillaries and edema between the collagen fibers of the skin dermis, along with bleeding in the layers of the basophilic cells (hair follicle bulbs), and leakage of some polymorphonuclear leukocytes. Lastly, there was bleeding in the wall of the hair follicles and loosening of the dermal collagen fibers (figure 10) This is consistent with what he said (Sakamoti and Nagao 2023)On the use of mice as a model of typical dermatitis Compared with the control group (figure 6)



**figure 6:** A photo of an 18-day-old mouse embryo from the control group shows the blood vessels, capillaries in the skin, the appearance of dermal fibers, sweat glands, and sebaceous glands in their normal form, along with the dilation of some sebaceous gland ducts. Hematoxylin and eosin stain 10X.: B Histological section of the gland of showing mild reaction as blue color of the collagen fibers (arrows). Masson Trichrome stain, 400X.

**figure 7:** A picture of an 18-day-old fetus rat, exposed to a dose of 400 mg/kg, shows edema around the hair follicles and between the collagen fibers of the dermis layer, as well as around the sweat and sebaceous glands. There is also edema between the skin and dermis layer, congestion of the blood vessels, and separation of the collagen fibers in the skin dermis. Hematoxylin and eosin staining 10X.

**figure 8:** A picture of an 18-day-old fetus of a mouse, pregnant with a dose of 400 mg/kg, shows atrophic degeneration in the spinous cell layer of the skin, along with edema in the deeper dermal layers and slight infiltration of leukocytes. It is expected that this area is inguinal, with congestion and slight bleeding in the urinary bladder. Additionally, the pelvic bones are still in the form of cartilage and have not completed ossification. Hematoxylin and eosin staining 10X.



**figure 9:** A photo of an 18-day-old fetus rat, conceived from a group of 600 mg/kg, illustrates hyperkeratosis of the skin's cornified layer, the presence of swelling (edematous shrinkage) in the prickly cell layer of the skin, loosening of the white fibers of the dermis, and congestion of the capillaries, stained with hematoxylin and eosin 10X. B. Histological section of the gland of showing mild reaction as blue color of the collagen fibers (arrows). Masson Trichrome stain, 400X.

**figure 10:** Image of an 18-day-old mouse fetus, which was carried by a group of 600 mg/kg. The image shows congestion of capillaries and edema between the collagen fibers of the dermal skin, as well as the presence of bleeding in the layers of the patina corpas (hair follicles) with infiltration of some white blood cells of multiple nuclear shapes (polymorphs). There is also bleeding in the wall of the air follicles and loosening of the white fibers of the skin. The stain used was hematoxylin and eosin 10X. B: Histological section of showing mild reaction as blue color of the collagen fibers (arrows). The amount of collagen fibers decreases, and the ratio of mesenchymal cells to collagen fibers is very high, Masson's triple stain 400X.

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