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Ammi Majus Seed Extract Cardioprotective Effect Against Doxorubicin Cardiotoxicity in Mice

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Abstract

One of the most efficient anthracycline anti-cancer medications, doxorubicin, is used to treat lymphoid malignancies. Intercalation with deoxyribonucleic acid is a key component of its primary chemotherapeutic mode of action, which can ultimately lead to heart failure. Therefore, the objective of this study was to determine whether the ethanolic extract of *Ammi majus* had any cardioprotective effects against doxorubicin toxicity in mice. 48 mature Albino male mice were separated into six groups and distributed as follows: for 14 days, Group I: "negative control" received distilled water; Group II: mice received a single oral daily dose of 64 mg/kg of Ammi majus seeds extract; Group III: mice received a single oral daily dose of 128 mg/kg of Ammi majus seeds extract; and Group IV: "positive control" received a single dose of 2 ml/kg of distilled water. On day fifteen, the mice got an intraperitoneal dose of 15 mg/kg of Doxorubicin; they were sacrificed using anesthetic ether 24 hours later. Group V was given a single dose of 15 mg/kg of doxorubicin on day 15 after receiving 64 mg/kg/day of *Ammi majus*. Finally, group VI mice received 128 mg/kg of *Ammi majus*, and on day 15, they got a single dosage of 15 mg/kg of doxorubicin. To analyze malondialdehyde, creatine kinase-myoglobin binding, and creatine phosphokinase as indicators of cardiotoxicity, the blood was drawn from the preorbital sac. Data analysis revealed that mice pre-treated with different doses of *Ammi majus* extract (64 mg and 128 mg/kg) significantly reduced the heart damage as compared to animals intoxicated by Doxorubicin, as evidenced by an increase in malondialdehyde, creatine kinase-myoglobin, and creatine phosphokinase in the Doxorubicin group. So the alcoholic extract of *Ammi majus* seeds reduced the heart injury in pretreated mice, because of its active constituent, which has anti-inflammatory action and antioxidant properties.

Keywords : *Ammi majus* doxorubicin, malondialdehyde, creatine phosphokinase, creatine kinase-myoglobin binding.

1. Introduction

Doxorubicin (DOX) is a member of the highly efficacious anti-cancer medication class of anthracyclines. It can be used alone or in conjunction with other chemotherapy-based treatments to treat a variety of adult and pediatric malignancies, including Hodgkin's and non-Hodgkin's lymphomas, neuroblastomas, soft tissue sarcomas, and solid tumors like breast cancer [1].

The mechanism of its cytotoxicity may entail the particular intercalation of the planar anthracycline nucleus with the DNA double helix, which might hinder further DNA replication [2] and, as a result, cause cellular survival-critical macromolecule synthesis to be inhibited and to cause DNA damage [3]. The reduction of doxorubicin to an unstable metabolite (semiquinone), which is then converted back to doxorubicin in a process that releases reactive oxygen species, may also be another way that doxorubicin affects cancer cells. This process results in the production of free radicals, which in turn cause damage to cellular membranes, DNA, and proteins. Reactive oxygen species (ROS) trigger the apoptotic pathways that lead to cell death, DNA damage, oxidative stress, lipid peroxidation, and membrane damage [4]. Furthermore, topoisomerase-II is negatively impacted by DOX when it enters the nucleus, which also causes DNA damage and cell death [5].Unfortunately, toxicities like hematopoietic suppression, myelosuppression, mucositis, nausea, vomiting, extravasation, alopecia, and cardiovascular adverse effects like heart failure, tachycardia, hypotension, and arrhythmias hampered its successful usage. However, cardiotoxicity is the most concerning side effect [6].*A. majus* (family Umbelliferae) is a traditional medicinal herb whose seeds are known as "Khella shaitani", This herb's seeds and roots have long been used to treat inflammatory disorders such as rheumatoid arthritis, as analgesics in the treatment of headaches, diaphoretics, and many other diseases [7]. Many pharmacological studies on *A. majus* have been conducted, and various therapeutic effects have been reported. Due to the enormous pharmacological effects of coumarins, much research has been conducted on them in recent years. Its chemical constituents among other substances, contained amorphous glucosides, tannins, oleoresins, acrid oils, fixed oils, proteins, and cellulose. Xanthotoxins, imperatorin, bergaptens, marmesin, isoimperatorin, heraclenin, and isomepimpinellin are the [8, 9] major constituents of furanocoumarins. Nonfurocoumarin, umbelliprenin, quercetin glycosides, luteolin, kaempferol, oleic acid methyl ester, palmitic acid, and additionally, linolenic acid were found [10–20]. It has many pharmacological properties, like antioxidants. An experimental investigation showed that crude extract had antioxidant activity as measured by free radical scavenging by DPPH [21, 22]. Gram-positive bacteria like Staphylococcus aureus (*S. aureus*) and Gram-negative bacteria like Escherichia coli (E. coli), *Haemophilus influenzae* (*H. influenzae*), and Proteus spp. (*Proteus* spp.) are all susceptible to the antibacterial effects of crude extracts of *A. majus* [10, 23]. Therefore, in addition to its widespread usage to control menstruation and as a diuretic, the effect of all crude extracts of *A. majus* calls for research to identify possible anticancer agents [19, 24, 25]. Antihyperlipidemic *A. majus* seed extract dramatically elevates high-density lipoprotein levels while considerably lowering triglyceride, cholesterol, and low-density lipoprotein levels [26]. The aim of the study was to determine the possible cardioprotective effect of *A. majus* seed extract on doxorubicin cardiotoxicity.

2. Materials and Methods

2.1. Plant material

In the Iraqi region of Baghdad, a local herbal market sold 1 kilogram of dried *A. majus* seeds. The plant was authenticated by assistant professor Dr. Ibrahim Salih, a botanist at the Department of Pharmacognosy, College of Pharmacy, University of Al-Mustansira, and Baghdad, Iraq. 1 kg of dried *A. majus* seeds are crushed and ground by a mortar before being continuously extracted (Soxhlet) with 1500 ml of n-hexane until the yellowish tint has vanished. The oil-free residue, which represents the defatted *A. majus* seeds, is extracted with 2 liters of 80% ethanol using the reflux method for 6 hours at 40 °C. After allowing the mixture to cool, filter paper is used to

remove the impurities. An extract devoid of ethanol and containing the active component found in *A. majus* seeds is produced by rotating vacuum evaporation of the filtrate at a temperature of 40 °C [27].

2.2. Experimental model

The present study was carried out on 48 albino male mice weighing 25–30 g and aged 8–10 weeks, obtained from the animal house of the College of Pharmacy, University of Baghdad, Iraq. The mice were kept in plastic cages (20*30 cm) with eight mice per cage. They were kept under conventional laboratory conditions, which included a temperature range of 22–24℃ and a 12-hour light/dark cycle, and they were offered free food access (commercial mouse pellets) and water. The experimental group kept acclimated for 5 days before starting the experiment.

2.3. Methodology

Mice were divided into six equal groups as follows to examine the potential preventive impact of various dosages of *A. majus* extract against DOX-induced heart damage:

Group I: 8 mice given for 14 days with a single oral dose of 2 ml/kg/day D.W. The group acted as the negative control.

Group II—8 mice were provided with a single oral dose of 64 mg/kg of the extract from *A. majus* seeds every day for 14 days.

Group III consists of 8 mice that were given a single oral dose of 128 mg/kg of *A. majus* seed extract daily for 14 days.

Group IV: 8 mice were given a single oral dose of 2 ml/kg/day D.W. for 14 days. The animal was given a single intraperitoneal injection of 15 mg of DOX on day 15 to cause a heart injury. The group acted as a positive control.

Group V-8 mice were treated with a single oral dose of 64 mg/kg/day of seed *A. majus* extract for 14 days, and then on the 15th day, the mice were given a 15 mg/DOX IP dose, 24 hours after DOX injection. The animals were sacrificed using anesthetic ether.

Group VI-8 mice received a single oral daily dose of 128 mg/kg/day of the extract from *A. majus* seeds beginning 14 days before receiving 15 mg/DOX at the 15-day mark. 24 hours after DOX injection, the animals were sacrificed using anesthetic ether.

Blood was collected after 24 hours of the last dose of distil water in group I, after 24 hours of the last dose of *A. majus* in groups II and III, and after 24 hours of the doxorubicin dose in groups IV, V, and VI by a heparinized microcapillary tube through the retroorbital sac. The drained blood (1–1.5 ml) was collected in the Eppendorf tube for 20 minutes until blood coagulation occurred. Serum was collected after centrifugation in a cold centrifuge at 3000 rpm for 20 minutes at 4°C and kept frozen for analysis.

2.4. Histopathological Examination of Heart Tissue:

The hearts of each mouse were carefully removed, washed in PBS solution (pH 7.4), and then taken and firmed with 10% formaldehyde solution for histological investigation after each mouse was put down by the anesthetic diethyl ether and by cervical dislocation, where the heart tissues were prepared for histological analysis by the histopathologist Dr. Salem Rasheed Al-Obaidi according to the method of Junqueira LC et al. (1995) using the paraffin sections technique [28]. The fixative tissue was dried, then cleaned with xylene to remove alcohol and give the heart tissue some transparency. The tissues were then saturated with paraffin wax, heated, and blocked by adding embedded models. The tissues were cut by microtoning into 5 m-thick sections, washed in a water bath, and left in the oven to finish the dewaxing process. Then it was stained with hematoxylin and eosin (H&E) dye, and the histopathologist viewed it under a light microscope.

3.Results

3.1.Effects of the Various Treatments on Heart Tissue Homogenate Malondialdehyde (MDA) Contents:

Table 1 showed that male mice IP injected with 15 mg/kg doxorubicin hydrochloride (Group IV) showed a significant increase (P< 0.05) in heart tissue homogenate of MDA compared to the corresponding activity in negative control animals (Group I), with the serum activity of the intended isoenzyme being (3.05 ± 0.96 vs. 1.29± 0.13), respectively. Moreover, table 1 showed that pre-treatment of mice with either 64mg (Group V) or 128mg/mice (Group VI) of *A. majus* extract for 14 days before doxorubicin had a significant reduction (p<0.05) in the heart tissue homogenate MDA activity in mice as compared with the corresponding activity level in positive control animals (Group IV). The Mean±SEM of serum MDA activities were (0.89 ± 0.14, 0.94±0.12 vs. 3.05 ± 0.96), respectively. Also, there were non-significant differences in the heart tissue homogenate of MDA groups of pretreated mice with 64 mg/kg (group V) and 128 mg/kg (group VI) of *A. majus* compared to the negative control mice group (group I). Mean±SEM of the serum activities of MDA were, respectively, (0.89±0.14, 0.94±0.12 vs. 1.29±0.13).Also, Table 1 shows that there is a non-significant difference in the homogenate heart tissue MDA activity between the pretreated mice with 64 mg/kg (group V) and 128 mg/kg (group VI) of *Ammi majus.*

Table 1: Effects of treatment with different doses of *Ammi majus* extract prior to DOX on the heart contents of MDA compared to DOX- treated and control groups

Groups	NO.	Treatments	MDA (mmole/L)
Group I	8	DW	1.29 ± 0.13^{b}
Group II	8	64 mg/Kg Ammi majus	0.96 ± 0.04^{b}
Group III	8	128 mg/Kg Ammi Majus	1.09 ± 0.07^{b}
Group IV	8	14 days DW prior to a single dose of doxorubicin	3.05 ± 0.96^{a}
Group V	8	64mg/Kg Ammi Majus prior to a single dose of doxorubicin	0.89 ± 0.14^{b}
Group VI	8	128/ Ammi Majus prior to a single dose of doxorubicin	0.94 ± 0.12^{b}

- Each value represents the mean ± standard error of means (SEM).
- (a, b)= Different superscripts indicate significant differences between designed groups (P<0.05) using an unpaired Student t-test. a- positive control group (Doxorubicin-treated animals) and b-other groups.- Values with the same letter superscript (b) are non-significantly different (P>0.05) among (I, II, III, V and VI) groups using ANOVA and LSD analyses.

3.2. Effect of various treatments on serum creatine phosphokinase levels in the experimental male mice:

Table 2 showed that there was a significant elevation (P<0.05) in the serum activity of the creatine phosphokinase (CPK) enzyme in the group of mice IP treated with a single dose of Dox (Group IV) as compared to the negative control (Group I). Pretreated mice with either 64 mg/kg (group V) or 128 mg/kg (group VI) of *A. majus* showed a significant reduction (p<0.05)in serum levels of CPK as compared to the positive control. Moreover, there were no significant differences between the dosage rates used for *A. majus***.** Furthermore, pre-treated mice with 64 mg/kg and 128 mg/kg of *A. majus* for 14 days before doxorubicin (Groups V and VI) showed a non-significant reduction (p>0.05) in serum levels of CPK as compared with the negative control (Group IV). Mean±SEM of the serum activities of CPK enzyme levels were (157.85±8.49 and 153.42±2.86 vs. 128.57±6.24), respectively.

Table 2 Effects of treatment of mice with different doses of *A majus* extract on the serum activities of CPK prior to DOX; compared to DOX treated and control groups:

Groups	Treatment	CPK, (IU/L) Mean ± SEM
Group I	DW	128.57±6.24[c]
Group II	64 mg/Kg *A majus*	109.85±4.61[c]
Group III	128 mg/Kg *A majus*	117.57± 2.58[c]
Group IV	14 days DW prior to a single dose of doxorubicin	216.42±10.20[a]
Group V	64mg/Kg *A majus* prior to a single dose of doxorubicin	157.85±2.86[b]
Group VI	128/ A majus prior to a single dose of doxorubicin	153.42±8.49b

3.3. Effects of various treatments on serum activities of creatine kinase-myoglobin binding (CK-MB) enzyme.

Table 3 demonstrated that the blood activity of the CK-MB enzyme was significantly elevated (9P<0.05) in the group of mice IP treated with a single dose of Dox (Group IV) as compared to the negative control (Group I). When compared to the positive control, pretreated mice with either 64 mg/kg or 128 mg/kg of *A. majus* (groups V and VI) displayed a substantial decrease (p<0.05) in serum levels of CK-MB. And there were no discernible differences between groups given various *A-majus* dosage rates. Furthermore, pre-treated mice with 64 and 128 mg/kg. for 14 days only (Groups II and III) showed a significant (p<0.05) reduction in serum levels of CK-MB as compared with the positive control (Group IV). Additionally, table (3) demonstrated a significant decrease (p<0.05) in the heart's serum CK-MB activity in the groups that received either 64 mg (Group II) or 128 mg/mice (Group III) of ***A majus*** extract for 14 days, as well as pretreated groups of mice that received 64 mg/kg (Group V) or 128 mg/kg (Group VI) of ***A. majus*** for 14 days prior to doxorubicin, when compared to the corresponding activity level in positive, The Mean±SEM of serum CK-MB activities were (88.66± 1.42, 88.33±1.30, 122.00±1.31,120.16 ±1.19, vs164.16±7.12.), respectively.

Table 3: Effects of treatment of mice with different doses of *A majus* extract on the serum activities of CK-MB prior to DOX; compared to DOX-treated and control groups:

Groups	Treatment	CK-MB, (IU/L) Mean ± SEM
Group I	DW	93.50±1.40[c]
Group II	64 mg/Kg *A majus*	88.66±1.42[c]
Group III	128 mg/Kg *A majus*	88.33± 1.30[c]
Group IV	14 days DW prior to a single dose of doxorubicin	164.16±7.12[a]
Group V	64mg/Kg *A majus* prior to a single dose of doxorubicin	122.00±1.31[b]
Group VI	128/ *A majus* prior to a single dose of doxorubicin	120.16±1.19[b]

[a-c] Different superscripts indicate significant differences between designed groups (P <0.05) using the unpaired Student t-test.

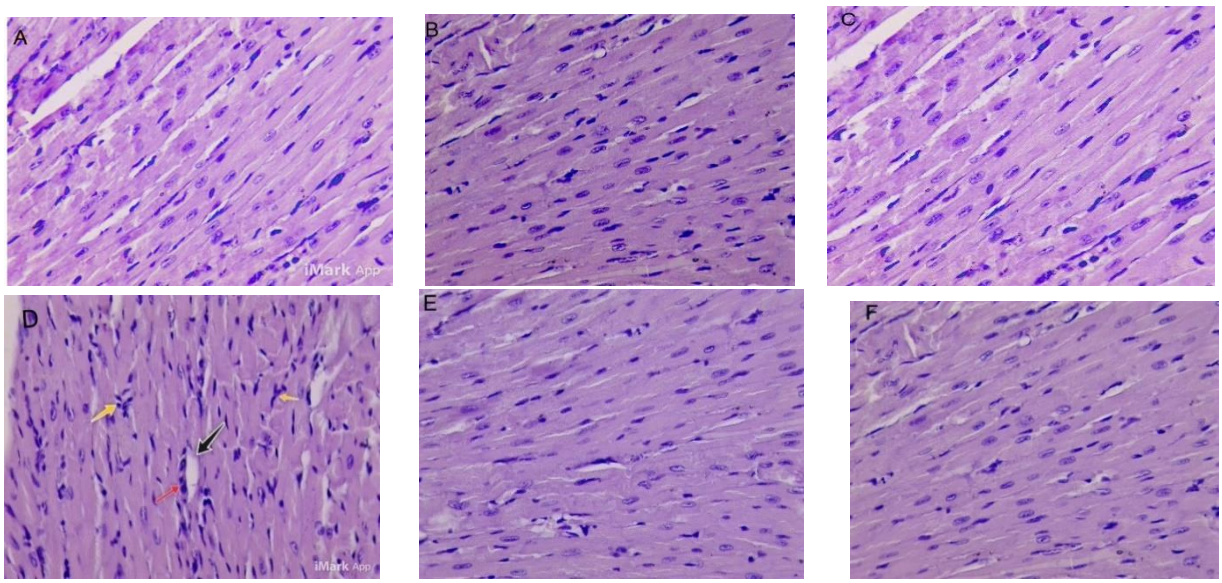

Figure 1. a: (Group I) negative control normal heart, B: Group II take *A. majus 64 mg*/kg with normal heart appearance; C: Group III take 128 mg/kg *A. majus* with normal heart appearance*;* Group IV: take 15 mg/kg doxorubicin; it has inflammatory cells and necrosis; Group V: group take 64 mg/kg of *A. majus* before doxorubicin looks normal; Group VI: take 128 mg/kg *A. majus* before doxorubicin looks normal.

4. Discussion:

A. majus is one of the plants that is particularly abundant in active components with antioxidant qualities, such as flavonoids like quercetin and kaempferol, and coumarin [19]; and these antioxidant qualities of *A. majus* extract have been identified when quercetin was used to treat diabetic nephropathy in rats [29]. This is consistent with the finding that the antioxidant activities of A. majus seed extract reduced the generation of free radicals by the chemotherapy drug doxorubicin. One of the byproducts of polyunsaturated fatty acid peroxidation in cells is malondialdehyde (MDA). Overproduction of MDA is brought on by an increase in free radicals. Malondialdehyde levels are frequently used as indicators of oxidative stress and the antioxidant state in cancer patients [30], and one of the main mechanisms linked to the toxicities of DOX-organic compounds is the excessive generation of reactive oxygen species (ROS) [31]. The results of the current study exhibit that the cardiac tissue MDA contents were significantly increased ($P<0.05$) in Dox-treated mice (Group IV) compared with negative control group I [table 1], and one of the main mechanisms linked to the toxicities of DOX-organic compounds is the excessive generation of reactive oxygen species (ROS) [31-33]. Additionally, the findings of this study demonstrated that 64 mg/kg and 128 mg/kg of *A. majus* (Groups V and VI, respectively) given before the 15 mg/kg Dox resulted in a significant reduction in MDA levels when compared to the positive controls (Group IV), which is related to its antioxidant action. In addition to quenching alkyl radicals to stop chain reactions, quercetin also has iron-chelating properties similar to ferritin's ability to bind iron during the Fenton reaction, which is another anti-oxidation mechanism[34]. It has been demonstrated that chronic oral therapy with quercetin (10 mg/kg/day) for five weeks lowers blood pressure, boosts glutathione production, and lowers levels of both plasma and hepatic malondialdehyde (MDA) [35, 36].The biomarkers CK-MB and CPK have been useful in the diagnosis of doxorubicin-induced cardiotoxicity because they are sensitive and specific indicators of myocardial injury [37]. A prior study found that after doxorubicin intraperitoneal 15 mg/kg (2.5 mg/kg body weight Ip) in 6 equal injections alternatively for 2 weeks, a total cumulative dose of 15 mg/kg body weight increased the serum CK-MB and CPK [31, 38–

40], and this is compatible with our result, which was characterized by a significant elevation ($P<0.05$) in serum creatine kinase myocardial bound CK MB isoenzyme and creatine phosphokinase CPK levels compared to the negative control group. It is well known that these enzymes are released from damaged heart muscle cells and that, following myocardial injury, blood levels of these enzymes serve as a measure of the severity of cardiac muscular damage. It was deemed to be a sensitive predictor with a decent correlation with the left ventricular systolic and diastolic functions. Moreover, the modification in the structure and function of the cardiomyocyte membrane caused by the binding of toxic Dox metabolites to the lipid and protein components of the membrane may be the cause of the high values of serum activities of both enzymes (CK-MB and CPK) [41]. In a model of Dox-induced myocardial damage, pretreatment with *A. majus* has been demonstrated to enhance cardiac function by lowering CPK and CK-MB expressions. Moreover, Bedouins advocate it as a healer plant in traditional medicine, and bioactive components like coumarin were studied. It was suggested that *A. majus* may have a lipid-lowering impact and/or an increased antioxidant status, which may have helped to lessen the doxorubicin tendency to elevate cardiac biomarkers. According to the data and results mentioned in tables 1 and 2, we notice that animals treated with *A. majus* restored the levels of CPK and CK-MB (groups V and VI) compared to a positive control (the doxorubicin-treated group), which indicates the extract's ability to lessen leakages of these enzymes from cardiomyocytes. Results of the current study showed that the *A. majus* doses alone (64 mg or 128 mg/kg) each produced non-significant differences in the CPK and CK-MB isozyme serum levels compared to the negative control (Group I). Thus, in the current study, prior to a single dose of doxorubicin, treatment with Ammi majus reduced the levels of the serum markers CPK and CK-MB. This could be attributed to *A. majus* 'protective effect on the myocardium by reducing the myocardial damage caused by Doxorubicin, which in turn limited the leakage of the intended markers into the serum, and this protective effect is due to its main ingredient, quercetin, which, when administered in a doxorubicin-induced heart injury model, protects the cardiomyocytes. By preventing cell apoptosis and maintaining cell shape by reorganizing the cytoskeleton, quercetin dramatically improves cell survival [36]. Moreover, there are other active ingredients with anti-inflammatory properties, such as the phytochemical coumarin [10], an antioxidant; the standard antioxidant gallic acid and the DPPH-H scavenging activities of plant crude extracts were quite similar; antioxidant action was attained due to the presence of phenols and flavonoids, which are thought of as secondary metabolites of plant origin [42]. Another study demonstrates the antioxidant properties and effects of the linear furanocoumarin marmesinin (an additional chemical component of *A. majus* on membranes [19]. Marmesinin isolated from aegle marmelose was evaluated during an experimental myocardial injury. Marmesinin oral treatment for 2 days before and during isoproterenol administration decreased the effect of lipid peroxidation. By preventing the release of beta-glucuronidase from the subcellular fractions, it was also demonstrated to have a membrane-stabilizing effect. As a result, marmesinin, a linear furanocoumarin, may offer protection against the harm brought on by experimental myocardial injury [43]. Additionally, the findings of a histological examination of a mouse heart before and after a doxorubicin toxicity test, as well as pre- and post-exposure to *A. majus* during the current study, are displayed in figure (1), which shows a normal heart in the control group and in the groups that take *A. majus* extract only. The positive group (the doxorubicin group) showed necrosis of cardiac myocytes, severe infiltration of inflammatory cells, mainly neutrophils, and severe vacuolation in the cytoplasm of the cardiac

muscles (myocytolysis), which returned to resembling normal cardiac architecture in groups receiving *A. majus* seed extract prior to doxorubicin (groups V and VI).

5. Conclusion:

We have concluded that *A. majus* seed ethanolic extract has a cardioprotective effect against doxorubicin cardiotoxicity.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethical Clearance

Ethics of scientific research were carried out in accordance with the international conditions followed in dealing with laboratory animals, and included animal health, husbandry and care for it, and providing appropriate conditions for it in terms of food, and appropriate methods were adopted in dealing with it when experimenting, and this is consistent with the instructions of the Iraqi Ministry of Health and Environment.

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