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Possible Cardioprotective Effect of Iraqi Ammi Majus Seed Extract on Doxorubicin Induced Cardiotoxicity in Mice

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Abstract

High-anticancer anthracyclines, including doxorubicin, have a long history of usage as chemotherapy drugs. However, its potent cardiotoxic side effects include dilated cardiomyopathy and congestive cardiac failure, which are dose-dependent, restrict its therapeutic usefulness. Due to the presence of various active ingredients like quercetin, marmesinin, kempefrol, and other substances that inhibit cytochrome p450 like xanthotoxin bergapten, imperatorin, and isoimpinellin, Ammi majus exhibits an antioxidant effect in diabetic nephropathy, myocardial injury. As a result, the objective of this study was to determine whether the seed extract of Ammi1 majus provided cardio-protection against DOX-induced heart injury in mice.

48 mature male mice were separated into six groups and distributed as follows: For 14 days, Group I—the "negative control"—mice received D.W. 2.Group II mice received a single oral dose of 64 mg/kg of Ammi1 majus seeds extract. 3.Group III mice received a single oral daily dose of 128 mg/kg of Ammi1 majus seeds extract. 4.Mice in the positive control (Group IV) group received a single oral dose of 2 ml/kg D.W. every day for 14 days. On day 15, the mice got a single IP dose of 15 mg/kg DOX. The mice were then terminated using anesthetic ether 24 hours later. 5. Group V were given a single dose of 15 mg/kg DOX on day 15 after receiving (64 mg/kg/day) of Ammi1 majus ethanolic extract for 14 days. 6. Finally, group (Group VI) mice received (128 mg/kg of Ammi1 majus seed extract for 14 days, and on day 15 they got a single dosage of 15 mg/kg DOX. The mice were then slaughtered 24 hours after receiving the DOX.

To analyze GSH, SOD, GPx, and catalase as indicators of cardiotoxicity, the hearts of mice were collected for the manufacture of tissue homogenate. Analysis of the data showed that mice pre-treated with different doses of Ammi1 majus extract (64 mg and 128 mg/kg/day for 14 days) significantly reduced oxidative stress as compared to group IV of animals intoxicated by DOX as demonstrated by a decrease in (GSH, SOD, GPx, and catalase) levels in heart tissue homogenate.

Keywords: Ammi1 majus, DOX,SOD, GSH, ROS.

Introduction:

A wide range of plant secondary metabolites are a valuable source of pharmaceuticals, agrochemicals, flavors, scents, colors, biopesticides, and food additives (Naik and Al-Khayri 2016). Appiaceae family like Ammi1 species contain biologically primary active compounds including coumarins and flavonoids (Al-Snafi 2013). There are numerous names that have been used to refer to this plant, including the Arabic names khillah and khillah shytani, the English term bishops weed, the Latin name amm1i, and the German

name amm1i (Hussein et al. 2016). Ammi1 majus is indigenous to Egypt, although it is also seen in the Kohaz Mountains, the Mediterranean Sea basin, West Africa, and some areas of Iran(Adham 2015). In Iraq, Ammi1 majus is typically found in fields, gardens, and by the sides of canals, frequently as a cultivated plant (Chakravarty 1976). Ammi1 majus contains four main linear furocoumarins (xanthotoxin, bergabten, imperatorin and isioimpiellin), the most pharmacological of which is xanthotoxin (Coimbra et al. 2012), which inhibit human liver CYP450 (Liu et al. 2020).Several enzymes, including glutathione S-transferase (GST), NAD (P) H quinone oxidoreductase, and aldehyde reductase, are activated by simple coumarins (Wiegand et al. 2009). Furthermore, they contain flavonoids with antioxidant and anti-tumor capabilities (quercetin and keampferol) (Wiegand et al. 2009). In chronic inflammatory animal models, Ammi1 majus extract also has anti-inflammatory properties, and the effect grew stronger with dose (Mishra et al. 2020).Viruses that infect mammals, such as the herpes simplex virus and the vesicular stomatitis virus (HSV), were inhibited by Ammi1 majus coumarins. By using the carrageenan-induced rat paw edema method, coumarins were proved for their ability to reduce inflammation. They possessed anti-inflammatory effects at a dose of 0.01 mg/100 g(Mishra et al. 2020).

Acute lymphoblastic leukemia is commonly treated with doxorubicin, a cytotoxic anthracycline antibiotic(El-Dakroory et al. 2014). The specific intercalation of the planar anthracycline nucleus to the DNA double helix may be the reason of its cytotoxicity. which can stop further DNA replication and, as a result, cause DNA damage and limit the synthesis of macromolecules that are necessary for maintaining cell life(Box 2007). Additionally, the production of free radicals and their resulting damage to cellular membranes, DNA, and proteins may be another way that doxorubicin affects cancer cells. This is because doxorubicin was first reduced to the unstable metabolite semiquinone, which is then converted back to doxorubicin in a process that releases reactive oxygen species (ROS)(Aniogo et al. 2017),(Jawad et al. 2019). Reactive oxygen species cause oxidative stress, DNA damage, lipid peroxidation and membrane damage, as well as activate apoptotic pathways that result in cell death (Mutlag 2012).

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 (Octavia et al. 2012).

So, the study's objective was to determine the possible cardioprotective effect of Iraqi *Ammi majus* seed extract on doxorubicin cardiotoxicity.

Materials and Methods:

Plant material:

1000gm of dried Ammi1 majus L. seeds are crushed and ground by a morter before being heated continuously extracted (Soxhlet) with 1500ml of n-hexane until the yellowish tint has vanished.

The residual, oil-free residue, which represents the defatted Ammi1 majus seeds, is extracted with 2 liters of 80% ethanol using the reflux method for 6 hours at 40 oC. The mixture is then allowed to cool and filtered through filter paper. An extract devoid of ethanol and containing the active component found in Ammi1 majus seeds is produced by rotating vacuum evaporation of the filtrate at a temperature of 40 °C (Hussain et al. 2012)(Muttlag et al. 2012).

Experimental model:
The present study was carried out on 48 mice, eight to ten weeks old, 25 to 30 g, received from the animal sanctuary of the College of Pharmacy at the University of Baghdad in Baghdad, Iraq.

Mice were separated into 6 groups of 8 and kept in plastic cages. They were kept under conventional laboratory conditions, which included a temperature range of 22–24°C and a 12-hour light/dark cycle, and they were offered free food access. (commercial mice pellets) and water. The research used 48 healthy male mice. After acclimating for three to five days, experimental mice will be randomly assigned into six groups of eight animals each.

Methodology:
Mice were divided into the following groups to examine the potential preventive impact of various dosages of Ammi1 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. On day 15, anesthetic ether was utilized to sacrifice the animals, and the blood will drown. The group acted as the negative control.

Group II—8 mice were provided with a single oral dosage of the extract from Ammi1 majus seeds every day for 14 days. On day 15, anesthetic ether was utilized to sacrifice the animals, and the blood will drown.

Group III consists of 8 mice that were given a single oral dose of 128 mg/kg of Ammi1 majus seed extract daily for 14 days. On day 15, the animals were killed using the anesthetic ether, and the blood was drowned.

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 15mg/DOX on day 15 to cause heart injury. The animals were sacrificed with anesthetic ether. The group acted as a positive control and blood was drawn 24 hours following DOX delivery.

Group V- 8 mice treated with single oral dose 64 mg / kg/day of seed of Ammi1 majus extract for 14 days then after on the day 15, the mice were given 15mg/DOX IP dose, 24 hours after DOX injection, the animals were slaughtered using anesthetic ether. Blood was drowned.

Group VI-8 mice received a single oral daily dose of 128 mg/kg/day of the extract from Ammi1 majus seeds beginning 14 days before receiving 15 mg/DOX at the 15-day . 24

hours after DOX injection, the animals were slaughtered using anesthetic ether. Blood was drowned.

After the animals were slaughtered using anesthetic ether, the hearts were rapidly removed, homogenized, and used to estimate the SOD, GPX, catalase, and GSH by ELISA analysis. After placing the Eppendorf containing the heart tissue next to an ice-filled beaker to keep it cold, the homogenizer machine was used to homogenize the tissue for one minute at speed 3. For 20 minutes at 4°C and 3000 rpm, the homogenate was spun in a cold centrifuge. The supernatant was removed using a micropipette and kept at -20 °C until it was time to analyze the parameters that would be utilized to gauge the cardiotoxicity brought on by DOX: SOD/GPX/catalase, and GSH.

Results:

The Effect Of Ammi1 Majus Seed Extract On The Heart Antioxidants Levels

Ammi1 majus alcoholic extract's anti-oxidant effects on ROS brought on by doxorubicin in an animal model are shown in (Table-1) and (Figure-1-4). The cardiac catalase1 levels in mice pre-treated with either 64 mg or 128 mg/mice of Ammi majus extract Group II& III respectively, for 14 days did not significantly increase ($P>0.05$) when compared to the negative control, but they did decrease significantly ($P<0.05$) in the doxorubicin-treated mice (Group IV) when compared to the negative control group (80.28±5.71 vs154.28±9.23 respectively). Pre-treated mice with 64 and 128 mg/mice concentrations of Ammi1 majus alcoholic seed extract before doxorubicin (Group V& VI), showed equivalent substantial increases ($p< 0.05$) on catalase1 level in treated mice in comparable with positive control (143.00±7.83 &144.73±12.93 vs 80.28±5.71 respectively) and this effect does not differ significantly from that produced by the negative control.

The findings on the impact of various doses of Ammi1 majus seed extract on cardiac GSH level revealed that doxorubicin-treated mice had significantly lower levels than the control group {(Table-1) (93.00±19.94 vs 234.71±18.55) and (Figure-2)}. When compared to mice treated with doxorubicin (93.00±19.94), animals pretreated with both dosages of Ammi1 majus—64 mg and 128 mg/kg (Group V, Group VI) —showed a considerable rise in GSH level (228.14±8.91)(231.00±10.15). Measurements of two additional antioxidants, glutathione peroxidases (GPxs) and superoxide dismutase (SOD), reveal a similar pattern of effect as pretreatment with 64 mg or 128 mg/kg/mice (Group V& VI), which resulted in statistically significant increases ($p<0.05$) in both GPX and SOD in pretreated mice with Ammi majus before doxorubicin (table1, figure3,4) as compared to mice that were treated with doxorubicin.

(Table-1): Effects of treatment of mice with different doses of Ammi majus extract on the activities of catalase, GSH, GPx and SOD prior to DOX compared to DOX-treated and control groups.

Groups	N	Catalase u/l Mean ±SEM	GSH mg/ml Mean ±SEM	GPx pg/ml Mean ±SEM	SOD pg/ml Mean ±SEM
Group I	8	154.28±9.23a	234.71±18.55a	71.96±2.18a	187.78±4.98a
Group II	8	158.57±4.47a	237.71±18.40a	71.53±3.97a	190.642±5.95a
Group III	8	163.71± 6.12a	239.14±16.55a	73.05±2.54a	192.00± 3.35a
Group IV	8	80.28±5.71b	93.00±19.94b	55.08±1.80b	88.42±5.21b
Group V	8	143.00±7.83a	228.14±8.91a	77.04±3.81a	181.71±3.89a
Group VI	8	144.73±12.93a	231.00±10.15a	77.85±1.73a	182±4.23a

Each value represents mean ± SEM; Group I= negative control, Group II=animals treated with 64mg/kg/day of Ammi1 majus seed extract, Group III= 128 mg/kg/day of Ammi majus-treated group, Group IV= 14 days/DW prior to a single dose of doxorubicin, animals; Group V=64mg/Kg Ammi1 Majus prior to a single dose of doxorubicin, and Group VI=128/kg Ammi Majus prior to a single dose of doxorubicin. Each value represents mean ± standard error of means (SEM).

- (a and b) = Different superscripts indicate significant differences between designed groups ($P < 0.05$) using unpaired Student t-test.

-Values with identical superscripts are significantly non-different ($P>0.05$) among (group I, II, III, V and VI) groups using ANOVA and LSD analyses.

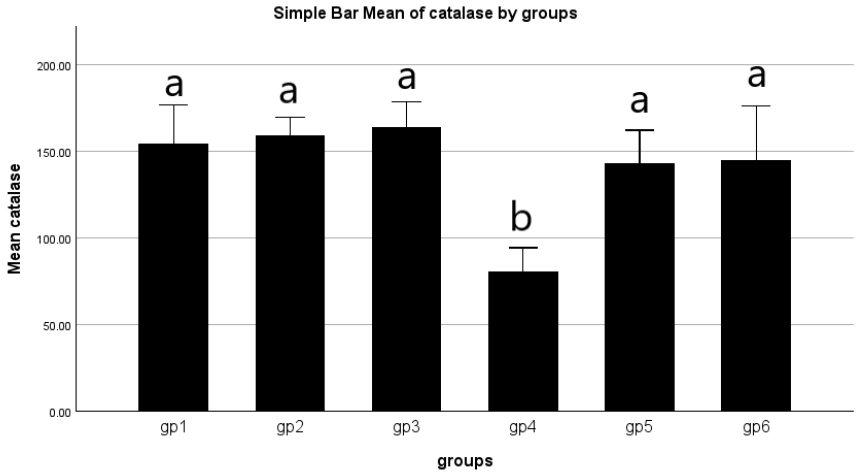

Figure (1) Mean increase in antioxidants (catalase) during experimentally induced cardiotoxicity of the study groups.

Figure (2) Mean increase in antioxidants (GSH) during experimentally-induced cardiotoxicity of the study groups.

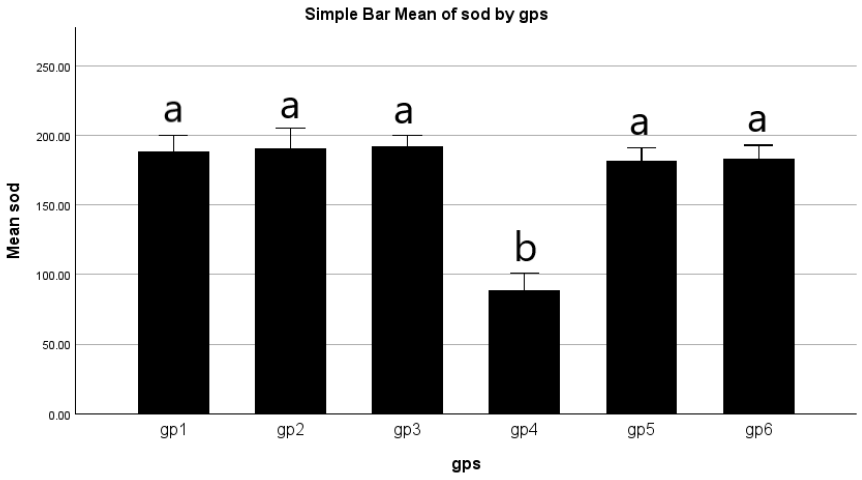

Figure (3) Mean increase in antioxidants (SOD) during experimentally-induced cardiotoxicity of the study groups.

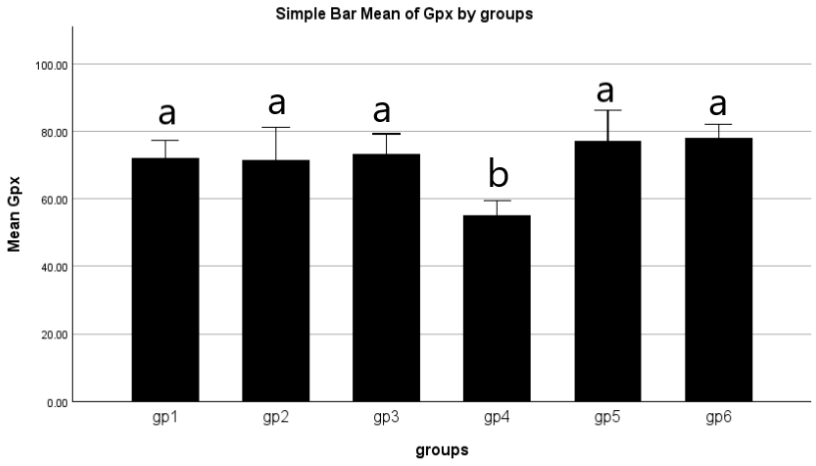

Figure (4): Mean increase in (GPX levels) during experimentally induced cardiotoxicity of the study groups.

Discussion:

The results derived from this research clearly illustrated the state of oxidative stress triggered in heart tissues by doxorubicin, expressed by a severe depletion of catalase1, GSH, GPx, and SOD contents in heart tissue homogenate in doxorubicin group (Group IV) as compared to negative control group (Group I) as shown in tables 1; these findings are consistent with those observed by others (Abdulrazzaq 2007),(Thandavarayan et al. 2015). The state of oxidative stress is exacerbated by reductions in glutathione levels, an essential water-soluble antioxidant that can straight scavenge reactive species developed during metabolism of doxorubicin in cardiomyocytes. Additionally, as more GSH was consumed for the conjugation of metabolites, the tissue's redox potential was compromised.

Notably, under conditions of oxidative stress, free radicals, expand because there is an imbalance between them and these defense mechanisms (Ali et al. 2021). The damage caused by ROS to cell macromolecules causes the cardiac tissue to malfunction. It also demonstrated that the production of free radicals, rises in cells with mitochondrial damage; in this regard, doxorubicin can enhance the production of free radicals, by impairing mitochondrial function (Lubrano and Balzan 2015). Additionally, this chemotherapy drug has the ability to lower GPx, GSH, SOD, and catalase levels in heart cells, which results in cell membrane destruction and dysfunction (Mutlag et al. 2011).

Increased lipid peroxidation led to bio-membrane degradation, which in turn adversely affected the permeability of the plasma membranes. The onset and progression of doxorubicin-induced cardiac dysfunction are mostly influenced by the oxidative stress driven on by increased ROS production(Das et al. 2011). Numerous investigations have shown that doxorubicin lowers myocardial antioxidants, interferes with adrenergic function, alters calcium homeostasis, and generates inflammatory cytokines, which

together lead to cardiac oxidative stress and cardiotoxicity(Li et al. 2008),(Mitry and Edwards 2016). The suppression of mitochondrial complex I and an inflammatory processes are also suggested to contribute to superoxide production in doxorubicin-induced heart injury. In light of this, important diseases that contribute to doxorubicin-induced cardiotoxicity include oxidative stress and inflammation.(Akolkar et al. 2017),(Su et al. 2019).

Ammi majus is one of the plants with an especially high concentration of active antioxidants, including flavonoids like quercetin, kaempferol, and coumarin(Al-Snafi 2013). And when quercetin was employed to treat diabetic nephropathy in rats, these antioxidant properties of Ammi majus extract were discovered (Anjaneyulu and Chopra 2004) and this agrees with the discovery that the antioxidant properties of Ammi majus seed extract decreased the production of free radicals by the chemotherapeutic medication doxorubicin.

In the present study, the groups that pretreated with Ammi majus seed extract dramatically boosted the antioxidant defense system, involving Catalase, SOD, GSH and GPX, in the hearts of mice treated with doxorubicin to suppress oxidative stress this is clear in groups that took 64mg/kg and 128mg/kg of Ammi majus seed extract Group V and Group VI respectively as compared to negative control (Group I) as clear in table1.

According to previous studies, the active ingredients in Ammi majus extract, including imperatorin (Piao et al. 2004),(Ng et al. 2000), bergapten (Oliveira et al. 2009)(Hu et al. 2019), isoimperatorin (Muttlag et al. 2012), and isopimpinellin, have potent antioxidant effects. One of the crucial elements that could stop the lipid peroxidation that gentamicin-induced lipid peroxidation in rats is the effect of Ammi majus extract on GSH levels (Koriem et al. 2012). Both directly and indirectly, reduced glutathione has strong antioxidant effects. The inflammatory response, brought on by doxorubicin was also lessened in mice given Ammi majus (Pieniazek et al. 2018). These active ingredients in Ammi majus, may assist in understanding the extract's anti-inflammatory properties.

The most prevalent flavonoid that scavenges both reactive oxygen species (ROS), and reactive nitrogen species(RNS) is quercetin. It may be utilized to lessen inflammation as well as oxidative stress, which is an imbalance between the formation of reactive species and their defense against them, which confirm its antioxidant activity in a number of studies, such as this one, which demonstrates the protective mechanisms for quercetin's action against doxorubicin-induced cardiomyocyte damage. By controlling metabolic activity, protein folding, and cytoskeleton rearrangement, quercetin may encourage cardiomyocytes to repair damage following doxorubicin treatment(Chen et al. 2013).

Also quercetin, was shown to have a protective effect against oxidative state in spontaneously hypertensive rats by Juan, D., and Milagros, G. Quercetin was chronically administered orally at a dose of 10mg/kg/day. Blood pressure is lowered and glutathione activity is increased after a five-week quercetin regimen(Galisteo et al. 2004).

Another study demonstrated the impact of Azdirachta indica leaf extract, which contains quercetin and kaempferol, on the level of glutathione-dependent enzymes and superoxide dismutase activity in the liver. A fixed dose of 500 mg/kg/day of the leaf extract administered for seven days in a row demonstrated an improvement in glutathione peroxidase, S-transferase, and superoxide dismutase activities.(Chattopadhyay 2003).

The mechanism of antioxidant action of flavonoids was not related to the direct scavenging activity only, but due to an increase in both the tissue GSH synthesis(Scharf et al. 2003) , and GSH antioxidant enzymes GPX and GR(Nagata et al. 1999)(Erden Inal and Kahraman 2000), which are in turn lead to increase the GSH level and total thiol status inside the tissue.

Additionally, quercetin can prevent TF-kB activation, directly lowering the generation of cytokines through this transcription factor (Mutlag 2012). Extracts of Ammi. majus demonstrated strong biological activity, including antiviral, antibacterial, antioxidant, relaxing, and cardiovascular effects (Hossain and Al Touby 2020). Ammi majus1 pre-treatment considerably increases antioxidant levels due to its protective effect against DOX-induced oxidative stress damage(Lubrano and Balzan 2015) . When compared to various conventional models, all produced extracts had significant antioxidant activity, according to an evaluation of the antioxidant activity of all polarity plant extracts using a variety of in vitro and in vivo methods. Earlier antioxidant activity findings for the specific plant species were also included in the evaluation (Sritharan and Sivalingam 2021).The majority of the data among them stated that the most polar extract, such as methanol extract, produced antioxidant activity. However, earlier studies on the same species showed that nonpolar extracts had the strongest activity(Attyah and Ismail 2012).

Conclusion:

We have concluded that Ammi majus seed has a cardioprotective effect because it contains antioxidant property and anti-inflammatory.

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