



CARDIOVASCULAR TOXICITY OF CHLORPYRIFOS IN FEMALE ADULT RATS: A PROBABLE PROTECTIVE EFFECT OF PHYTOCHEMICAL ISOFLAVONES

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Chlorpyrifos (CF) an organophosphate insecticide broadly used for pest control and sterilization in agriculture applications. It has also established that CF toxicity linked to the increase of oxidative stress with accumulation of the lipid peroxides. CF poisoning causes the heart tissue to produce high quantity of free radicals, become sensitive to oxidative stress, and damage. Studies indicated that soy contains plenty of isoflavones, mainly genistein (GS), which have antioxidant properties. Current study designed to examine whether GS, daidzein (DZ), or their combination had any protection against CF induced cardiac toxicity in rats. Thirty adult female rats divided into five groups. Group 1 received corn oil. Group 2 received CF 6.7 mg/kg. Group 3 received 6.7 mg/kg CF + 21.7 mg/kg GS. Group 4 received 6.7mg/kg CF + 17.4mg/kg DZ. Group 5 received 6.7mg/kg CF + 21.7 mg/kg GS + 17.4 mg/kg DZ daily oral doses for six weeks. At the end, rats sacrificed and their blood samples and heart tissues collected for biochemical and histopathology analysis. Conclusion: administration of GS or DZ provides a protective effect against oxidative stress induced by CF. However, administration of GS and DZ together synergise oxidative stress induced by CF.

Key words: Chlorpyrifos, genistein, daidzein, heart histopathology, Troponin, MDA.

INTRODUCTION

Chlorpyrifos (CF) (O, O-diethyl 0-3,5,6-trichloro-2-pyridylphosphoro-thioate) an organophosphate (OP) insecticide broadly used for pest control and sterilization in agriculture applications¹. It has also established that CF toxicity is linked to the increase of oxidative stress with accumulation of the lipid peroxides in various organs² causes numerous health risks³. Cardiovascular system affected by OP poisoning, cardiovascular manifestations include hypertension, sinus tachycardia, and bradycardia and reduced heart rate⁴. CF poisoning causes the heart tissue to produce high quantity of free radicals, become sensitive to oxidative stress, and damage⁵, these effects causes cardiovascular changes include an increase in cardiac marker enzymes level in serum; such as lactate dehydrogenase (LDH) and creatinine kinase-MB⁶. Because of CF

widespread use, its residues detected in crops and in air^{7&8}, which are considered dangerous to the living organisms⁹.

Traditional soy consumption associated with low incidence of cardiovascular diseases and cancers¹⁰. Genestein (GS) and daidzein (DZ) are the main isoflavone phytoestrogens in the soy products. GS found naturally in soybeans, chickpeas, lentils, sunflower seeds and other soy products¹¹. Studies indicated that soy contains plenty of isoflavones, mainly GS, which have antioxidant properties^{12&13}. GS has been stated to stop vascular remodelling and right ventricular hypertrophy¹⁴ Furthermore, GS is identified as modulator of Nitric oxide synthase (NOS) activities, the inhibition of NOS shown to avoid myocardial remodelling¹⁵. It indicated that genistein protects against doxorubicin-induced cardiotoxic effect by increase signalling of Nrf-2/HO-1¹⁶.

A study revealed that daidzein at a normal serum concentration bind to estrogen receptors on plasma membrane and stimulate synthesis of catecholamine, but at high concentrations decreases the acetylcholine – induced synthesis and secretion of catecholamine in the adrenal medulla of bovine and may be in sympathetic neurons. The later action of daidzein might be helpful on the cardiovascular system¹⁷.

Therefore, the current study designed to examine whether genistein, daidzein, or their combination had any protection against CF induced cardiac toxicity in rats.

MATERIALS AND METHODS

Materials

CF %ε^ TC (Al-Ardh Attyiba Co. for Manufacturing of Vet & Disinfectants, Amman - Jordan), diluted in corn oil to get a final concentration of 10% emulsion. GS (5,7-dihydroxy-8-[4-hydroxyphenyl]chromen-4-one) and DZ (4,7 Dihydroxyisoflavone) powders both from axenic research and formulation materials, China. GS and DZ were dissolved in distilled water.

Methods

Thirty adult Sprague-Dawley female rats with (weights of 170-200 gm) were involved in this experiment. The rats purchased from Hella University/ College of Science and acclimated for a period of one week before starting experiment. Each three animals caged in a plastic cage with standard bedding. Experiments of this study performed according to the National Institute of Health (86/609/EEC) Guidelines of using Laboratory Animals.

Furthermore, College of Pharmacy /University of Basrah / Ethical Committee approval number 3/5/115, September 2020 were obtained. The animals hosted in groups under suitable situations at room temperature of 25 ± 3 °C and humidity, with regular rhythm 12 h Light and 12 h dark. Animals served a pellet diet without soy and the drinking water supplied all the time.

Thirty adult female albino rats equally divided into five groups of six rats each. Animal's weights measured at the beginning and at the end of experiment. Group 1 rats represents the control group, administered corn

oil (1 ml /kg body weight)/ day orally by gavages. Group 2 rats administered orally CF 1/20th LD50 (6.7 mg/kg bw)/ day dissolved in 1ml corn oil and given orally by gavage. Group 3 rats administered (6.7 mg/kg bw CF + 21.7mg/kg bw GS) daily. Group 4 rats administered (6.7 mg/kg bw CF + 17.4 mg/kg bw DZ) daily. Group 5 rats administered (6.7mg/kg bw CF + 21.7 mg/kg bw GS + 17.4 mg/kg bw DZ) daily Doses were selected according to the previous researches.

After six weeks, the end of experiment period, the overnight fasted rats were sacrificed and then the blood samples were taken by intracardiac puncture and the sera collected by centrifugation at $855 \times g$ for 10 min. Sera were kept for biochemical tests at -20°C .

The heart from each rat removed and washed with saline solution (0.9%), then separated longitudinally into two parts and fixed in 10% formalin for histopathological examination.

Biochemical analysis

Serum level of troponin measured using Abbott ARCHITECT high-sensitivity troponin I assay (Abbott Diagnostics) employing chemiluminescent microparticle immunoassay (CMIA) method. While serum malondialdehyde (MDA) was measured using Bioassay Technology Laboratory (BT LAB) ELISA kit.

Histopathological examination

Histopathological examination was set by fixing the tissues in 10% formalin solution for about 4 days, later the tissues were prepared as sections, and covered by paraffin. The size of cut of histological pieces is 4 to 5 μm , stained by routine haematoxylin (H) and eosin (E) stains. After staining, the sections were examined under a light dissection microscope.

Statistical analysis

Statistics accomplished using Graph Pad Prism software (version 7.0, Inc., San Diego, CA). Descriptive data presented statistically as mean \pm SEM for wholly assessed parameters.

One-way analysis of variance (ANOVA) and Tuckey's Multiple Comparison tests used for comparison between groups. *p* values less than 0.05 regarded as significantly different.

RESULTS AND DISCUSSION

Results

The results of this study revealed no decrease in body weight of animals in the experimental groups throughout the study period and no change in overall appearance of animals reported. There was a significant body weights increase at the end of experiment in all groups except for CF group, as illustrated in figure (1).

Exposure to CF alone in a daily dose of 6.7 mg/kg bw for six weeks caused significant

increase in serum levels of troponin I ($p < 0.0001$) and MDA ($p < 0.001$). Whereas co-administration of GS with CF to female rats found to reduce the above changes, near the normal level. Co-administration of DZ with CF to female rats showed a significant increase in serum levels of troponin I ($p < 0.05$) but not MDA. While administration of GS, DZ and CF all together showed a significant increase in serum levels of troponin I ($p < 0.0001$) and MDA ($p < 0.001$) compared to control indicating a synergistic effect of soy isoflavones with CF, as illustrated in figure (2).

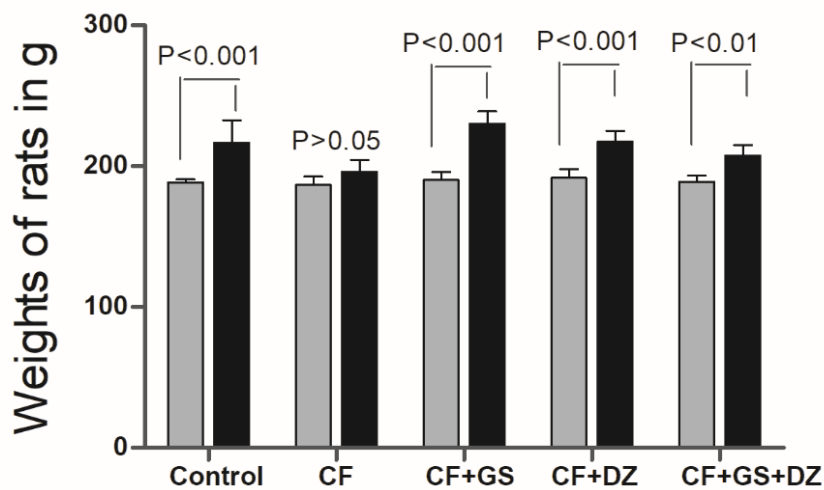


Fig. 1: Variations in the body weight of animals in different studied groups. All data presented as mean \pm SEM. Grey columns represent weights of rats at the start point of experiment while black columns represent weights of rats at the end after 42 days of experiment. CF (chlorpyrifos), GS (Genestein) and DZ (daidzein). $P < 0.05$ is significant difference

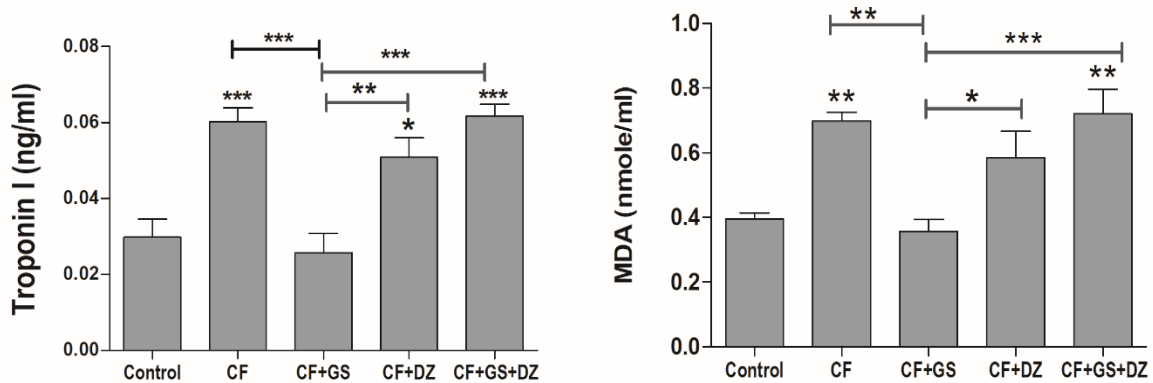


Fig. 2: Serum troponin I and MDA levels in different studied groups after administration of a daily doses of 6.7mg/kg bw CF (chlorpyrifos), 21.7mg/kg bw GS (Genestein) and 17.4mg/kg bw DZ (daidzein) for six weeks. All data presented as mean \pm SEM. (***) $p < 0.0001$, (**) $p < 0.001$ and (*) $p < 0.05$ in comparison to control.

Histopathological analysis of hearts

Control heart section of rats treated with only corn oil showed normal architecture structure of myocardium components such as myocytes, sarcolemma, and nucleus (figure 3-a). While, the heart section of rats treated with CF alone in a dose of 6.7 mg/kg bw for six weeks has shown a damage of myocyte and the affected area undergoes severe degeneration (myocytosis) with vacuolation of nuclei. As well as disruption of the sarcolemma, as shown in (figure 3-b). In contrast, the heart section of rats treated with 6.7 mg/kg bw CF + 21.7 mg/kg bw GS daily for six weeks disclose a recovery to normal function and normal myocyte, sarcolemma compared with CF alone

treated group, as shown in (figure 3-c). While, heart section of rats treated with 6.7mg/kg bw CF + 17.4 mg/kg bw DZ daily for six weeks, shows little myocardium fiber thickness with hemorrhage, and congestion in some area, with nucleus vacuolation, also, in some area the myocardium was appeared normal function, as shown in (figure 3-d). Heart section of rats treated with (6.7 mg/kg bw CF + 21.7 mg/kg bw GS + 17.4 mg/kg bw DZ) daily for six weeks, shows massive myocardium degeneration with thickness, nucleus vacuolation, and sarcolemma disrupted, as shown in (figure 3-e).

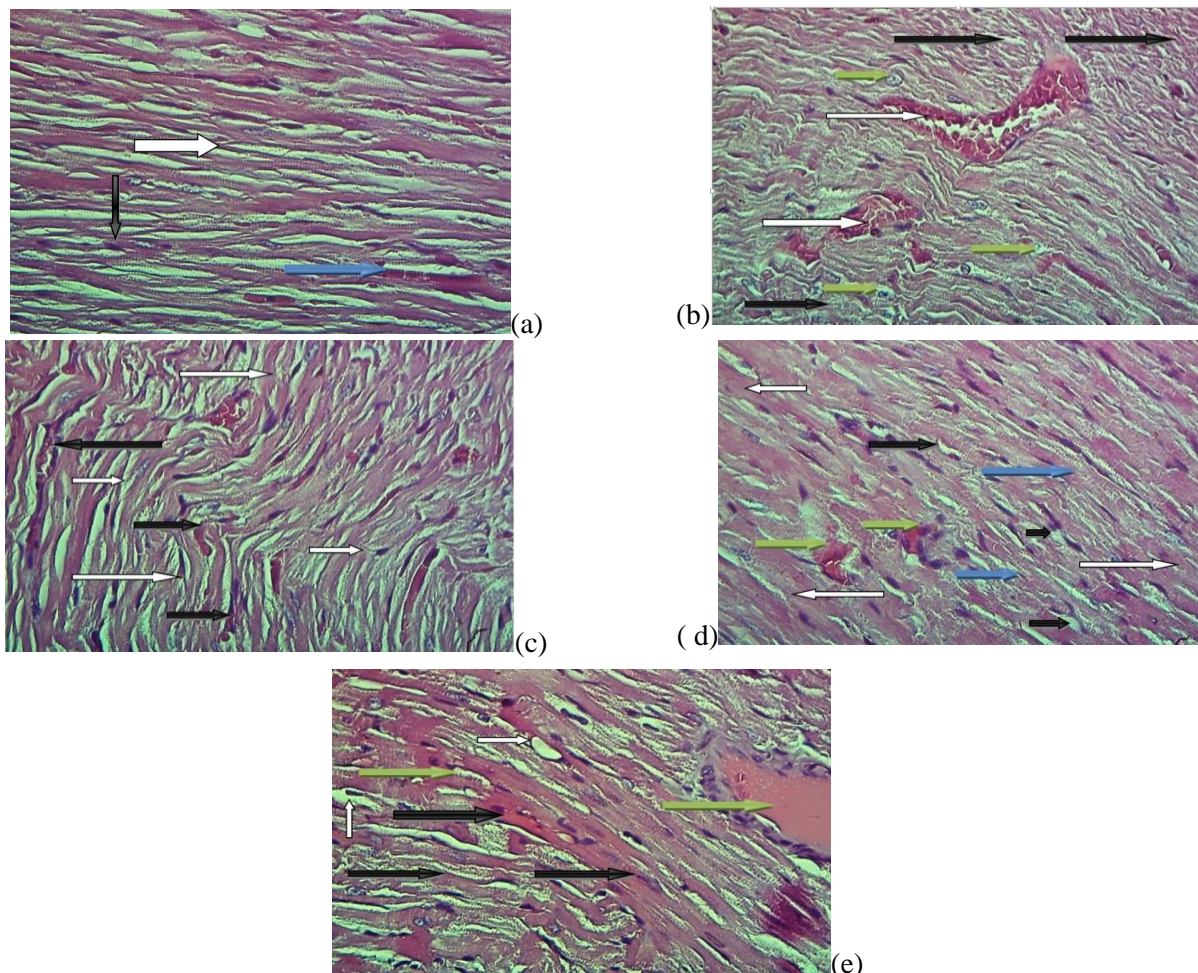


Fig. 3. Light micrograph of heart section from female rat (stained with H and E) 200X. **(a)** Control: showed normal myocardium architecture structure, striated myocyte, branches (white arrow), central nucleus (black arrow), and sarcolemma (blue arrow). **(b)** CF: shows massive degeneration in myocardium organs such as myocyte (black arrow), sarcolemma disrupted (white arrow), and nucleus vacuolation (green arrow). **(c)** CF + GS: shows normal myocyte (striate, branches) and nucleus (white arrow), normal sarcolemma respectively (black arrow). **(d)** CF + DZ: shows little myocardium fiber thickness with hemorrhage (white arrow), and congestion in some area (green arrow), with nucleus vacuolation (black arrow), in some area the myocardium appear normal (blue arrow). **(e)** CF + GS + DZ: shows massive myocardium degeneration with thickness (black arrow), nucleus vacuolation (white arrow), and sarcolemma disrupted (green arrow).

Discussion

Cardiac toxicity effects are the main cause of death in severe OP poisoning because of sudden development of ventricular fibrillation leading to cardiac death¹⁸. In the current study, it is revealed that exposure of rats to CF for six weeks caused a significant increase in serum troponin I that is extensively used as a marker for heart damage. This result is in cope with previous study shows a significant increase in troponin I in animals after exposure to dichlorvos, which is an OP compound⁷.

It was revealed that GS prevents cardiac hypertrophy induced by isoproterenol in rats¹⁹ and protects against Cardiac toxicity induced by doxorubicin in mice¹⁶. Furthermore, GS gives an effective reduction of postischemic depressed myocardial function and recovers myofibrillar responsiveness to Ca²⁺ in rats²⁰. In agreement, current study verified that simultaneous administration of GS to rats exposed to CF caused significant reduction in serum levels of troponin I and MDA, indicating GS cardiac protective effect.

Elevated oxidative stress is believed to be involved in CF cardiac toxic effects⁷; it is in cope with our current study verified increased oxidative stress, illustrated by significant increase in MDA level, signifying imbalanced redox case in the myocardial cells after CF exposure. This is in agreement with the current histopathological findings in heart tissue of CF exposed rats, characterized by degeneration and disorganization of cardiac fibers, connective tissue edema and vacuolization of cytoplasm, all these changes causes elevation of ROS in heart tissue⁶.

CF toxicity may include other mechanisms, not only oxidative stress. One of these mechanisms is induction of the major forms of cell death called apoptosis, which is distinguished by the losing of mitochondrial potential, fragmentation of nucleus and Bcl-2 down regulation²¹⁻²³. Other studies stated that induction of apoptosis in different organs by activation of caspase pathway²⁴. In the current study, CF alone in a dose of 6.7 mg/kg bw for six weeks has shown a damage of myocyte and the affected area undergoes severe degeneration (myocytosis) with vaculation of nuclei. As well as disruption of the sarcolemma, indicating cell cycle arrest and

induction of apoptosis in myocardial cells following CF exposure. The Induction of apoptosis may be caused by peroxidation due to ROS in the mitochondrial membrane, lead to the loss of the cell integrity, increase permeability of membrane and damage of DNA, which contribute to the death of cell²⁵.

However, there is now robust evidence about the ability of antioxidant supplements to prevent DNA fragmentation as well as apoptosis²⁶. The finding of this study were similar, CF administration with GS antioxidant agent causes a recovery to normal function and normal myocyte, sarcolemma compared with CF alone treated group.

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Conflicts of interest

The authors state that they have no conflicts of interest.

Ethical statement

All tests performed in agreement with the National Institute of Health Guidelines for the Treatment and Use of Laboratory Animals (86/609/EEC) and permitted by Basrah University, College of Pharmacy Ethical Committee.

REFERENCE

1. R. Lemus and A. Abdelghani, "Chlorpyrifos: an unwelcome pesticide in our homes", *Reviews on Environmental Health*, 15(4), 421-433 (2000).
2. M.D. Saulsbury, S.O. Heyliger, K. Wang, *et al.*, "Chlorpyrifos induces oxidative stress in oligodendrocyte progenitor cells", *Toxicology*, 259 (1-2), 1-9 (2009).
3. S.C. Joshi, R. Mathur and N. Gulati, "Testicular toxicity of chlorpyrifos (an organophosphate pesticide) in albino rat", *Toxicology and Industrial Health*, 23 (7), 439-444 (2007).
4. E.G. Smith and C.J. Gordon, "The effects of chlorpyrifos on blood pressure and

- temperature regulation in spontaneously hypertensive rats", *Basic & Clinical Pharmacology & Toxicology*, 96(6), 503-511 (2005).
5. B. A. Hatice and Y. Kalender, "Chlorpyrifos induced cardiotoxicity in rats and the protective role of quercetin and catechin", *Gazi University Journal of Science* 24 (3), 387-395 (2011).
 6. A. Bayır, H. Kara, Ö. Köylü, *et al.*, "The effects of ubiquinone (CoQ10) on heart tissue in cardiac toxicity related to organophosphate poisoning", *Human and Experimental Toxicology*, 32 (1), 45-52 (2013).
 7. M.A. Randhawa, F.M. Anjum, A. Ahmed, *et al.*, "Field incurred chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol residues in fresh and processed vegetables" *Food Chemistry*, 103(3), 1016-1023 (2007).
 8. M. Cattani, K. Cena, J. Edwards, *et al.*, "Potential dermal and inhalation exposure to chlorpyrifos in Australian pesticide workers", *Annals of Occupational Hygiene*, 45(4), 299-308 (2001).
 9. Q. Zhao, M. Dourson and B. Gadagbui, "A review of the reference dose for chlorpyrifos", *Regulatory Toxicology and Pharmacology*, 44(2), 111-124 (2006).
 10. F.M. Sacks, A. Lichtenstein, L. Van Horn, *et al.*, "Soy protein, isoflavones, and cardiovascular health: an American Heart Association Science Advisory for professionals from the Nutrition Committee", *Circulation*, 113(7), 1034-1044 (2006).
 11. W. Mazur, "11 Phytoestrogen content in foods", *Baillière's Clinical Endocrinology and Metabolism*, 12(4), 729-742 (1998).
 12. C.E. Park, H. Yun, E.B. Lee, *et al.*, "The antioxidant effects of genistein are associated with AMP-activated protein kinase activation and PTEN induction in prostate cancer cells", *Journal of Medicinal Food*, 13(4), 815-820 (2010).
 13. S. D. Abdulretha, A. S. Abdullah, M. S. AL Mozie'l," Phytochemical effects of genistein and daidzein on sex hormones and corticosterone in female adult rats exposed to Chlorpyrifos", *Toxicology and Environmental Health Sciences*, 8,1-7 (2022)
 14. N. Homma, Y. Morio, H Takahashi, *et al.*, "Genistein, a phytoestrogen, attenuates monocrotaline-induced pulmonary hypertension", *Respiration*, 73(1), 105-112 (2006).
 15. A.E. Valsecchi, S. Franchi, A.E. Panerai, *et al.*, "The soy isoflavone genistein reverses oxidative and inflammatory state, neuropathic pain, neurotrophic and vasculature deficits in diabetes mouse model", *European Journal of Pharmacology*, 650(2-3), 694-702 (2011).
 16. Z. Bai and Z. Wang, "Genistein protects against doxorubicin-induced cardiotoxicity through Nrf-2/HO-1 signaling in mice model", *Environmental Toxicology*, 34(5), 645-651 (2019).
 17. M. Liu, N. Yanagihara, Y. Toyohira, *et al.*, "Dual effects of daidzein, a soy isoflavone, on catecholamine synthesis and secretion in cultured bovine adrenal medullary cells", *Endocrinology*, 148(11), 5348-5354 (2007).
 18. S. Vijayakumar, M. Fareedullah, E.A. Kumar, *et al.*, "A prospective study on electrocardiographic findings of patients with organophosphorus poisoning", *Cardiovascular Toxicology*, 11(2), 113-117 (2011).
 19. S.K. Maulik, P. Prabhakar, A.K. Dinda, *et al.*, "Genistein prevents isoproterenol-induced cardiac hypertrophy in rats", *Canadian Journal of Physiology and Pharmacology*, 90(8), 1117-1125 (2012).
 20. J.Y. Min, H. Liao, J.F. Wang, *et al.*, "Genistein Attenuates Postischemic Depressed Myocardial Function by Increasing Myofilament Ca²⁺ Sensitivity in Rat Myocardium1", *Experimental Biology and Medicine*, 227(8), 632-638 (2002).
 21. B. M. Razavi, H. Hosseinzadeh, A.R. Movassaghi, *et al.*, "Protective effect of crocin on diazinon induced cardiotoxicity in rats in subchronic exposure", *Chemico-Biological Interactions*, 203(3), 547-555 (2013).
 22. A. S. Abdullah, H. M. Hameed, R. S. Baiwn, "Health risk evaluation of toxic polycyclic aromatic hydrocarbons (PAHs)

- in the street dust of Basra Iraq”, *Bulletin of Pharmaceutical Sciences Assiut University*, 45 (1), 469-479 (2021).
23. Y. Asari, Y. Kamijyo and K. Soma, "Changes in the hemodynamic state of patients with acute lethal organophosphate poisoning" *Veterinary and Human Toxicology*, 46(1), 5-9 (2004).
 24. S.C. Joshi, R. Mathur and N. Gulati, "Testicular toxicity of chlorpyrifos (an organophosphate pesticide) in albino rat" *Toxicology and Industrial Health*, 23(7), 439-444 (2007).
 25. R. Kapoor and P. Kakkar, "Naringenin accords hepatoprotection from streptozotocin induced diabetes in vivo by modulating mitochondrial dysfunction and apoptotic signaling cascade", *Toxicology Reports*, 1, 569 - 581 (2014).
 26. N. Galang, H. Sasaki and N. Maulik, "Apoptotic cell death during ischemia/reperfusion and its attenuation by antioxidant therapy", *Toxicology*, 148(2-3), 111-118 (2000).



نشرة العلوم الصيدلانية جامعة أسيوط



السمية القلبية الوعائية للكلوربيريفوس في إناث الجرذان البالغة: تأثير وقائي محتمل للأيزوفلافونات

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الكلوربيريفوس (CF) هو مبيد حشري من الفوسفات العضوي يستخدم على نطاق واسع لمكافحة الآفات والتعقيم في التطبيقات الزراعية. وقد أثبت أيضاً أن سمية CF مرتبطة بزيادة الإجهاد التأكسدي مع تراكم بيروكسيدات الدهون. يتسبب التسمم بـ CF في أن تنتج أنسجة القلب كمية كبيرة من الجذور الحرة، وتصبح حساسة للإجهاد التأكسدي والضرر. أشارت الدراسات إلى أن فول الصويا يحتوي على الكثير من الأيزوفلافون، وخاصة الجينيستين (GS)، والتي لها خصائص مضادة للأكسدة. الدراسة الحالية مصممة لفحص ما إذا كان GS أو DZ (daidzein) أو مزيجهما لهما أي حماية ضد تسمم القلب الناجم عن CPF في الفئران. ثلاثون أنثى بالغة من الجرذان مقسمة إلى خمس مجموعات. تلقت المجموعة الأولى زيت الذرة. تلقت المجموعة الثانية 6,7CF مجم / كجم. تلقت المجموعة الثالثة 6,7 3 مجم / كجم CF مع 21,7 ملجم / كجم GS وتلقت المجموعة الرابعة 6,7 ملجم / كجم CF مع 17,4 ملجم / كجم DZ وتلقت المجموعة الخامسة 6,7 ملجم / كجم CF مع 21,7 ملجم / كجم GS مع 17,4 ملجم / كجم DZ جرعات فموية يومية لمدة ستة أسابيع. وفي النهاية، تم التضحية بالجرذان وأخذ عينات الدم وأنسجة القلب للتحليل الكيميائي الحيوي وتشريح الأنسجة. والخلاصة إن إعطاء GS أو DZ يوفر تأثيراً وقائياً ضد الإجهاد التأكسدي الناجم عن CF. ومع ذلك، إن إعطاء GS وDZ معاً تآزر الإجهاد التأكسدي الناجم عن CF.