International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2023.321.328

Research Article
Potent Protective Effect of Lacidipine, Carvacrol and Lacidipine-Carvacrol Combination Against 5-Fluorouracil-Induced Cardiotoxicity in Rats

[1]Nergis Akbaş and [2]Halis Süleyman

[1]Department of Medical Biochemistry, School of Medicine, Erzincan Binali Yıldırım Üniversitesi, 24030, Erzincan, Türkiye
[2]Department of Pharmacology, School of Medicine, Erzincan Binali Yıldırım Üniversitesi, 24030, Erzincan, Türkiye

Abstract

Background and Objective: 5-Fluorouracil (5-FU) is the second most common cardiotoxic chemotherapeutic drug. Inflammation and oxidative stress are implicated in the pathogenesis of 5-FU-induced cardiotoxicity. This study evaluated the effectiveness of lacidipine and carvacrol, which have anti-inflammatory and antioxidant properties, against 5-FU-induced cardiotoxicity. **Materials and Methods:** Rats were divided into a control group (HC) and groups receiving 5-FU (FU), 5-FU+lacidipine (LFU), 5-FU+carvacrol (CFU), or 5-FU+lacidipine+carvacrol (LCFU). Malondialdehyde (MDA), Glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and Interleukin-6 (IL-6) from heart tissue and Creatine Kinase MB (CK-MB) and troponin I (TP-I) from plasma were evaluated. **Results:** The SOD and CAT activities and GSH levels were significantly lower in the FU group than HC, LFU, CFU and LCFU groups ($p<0.001$). The MDA, IL-6, TP-I and CK-MB levels were significantly higher in the FU group than HC, LFU, CFU and LCFU groups ($p<0.001$). Differences between HC and LCFU groups were detected only in terms of GSH ($p = 0.09$). **Conclusion:** The lacidipine and carvacrol are effective against 5-FU-induced cardiotoxicity, but a combination is more effective.

Key words: Cardiotoxicity, carvacrol, fluorouracil, inflammation, lacidipine, oxidative stress

Citation: Akbaş, N. and H. Süleyman, 2023. Potent protective effect of lacidipine, carvacrol and lacidipine-carvacrol combination against 5-fluorouracil-induced cardiotoxicity in rats. Int. J. Pharmacol., 19: 321-328.

Corresponding Author: Nergis Akbaş, Department of Medical Biochemistry, School of Medicine, Erzincan Binali Yıldırım University, 24030, Erzincan, Türkiye
Tel: +90 5333595361 Fax: +90 4462122211

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The 5-Fluorouracil (5-FU), an antitumour agent that is also known as the capecitabine metabolite, was first introduced in 1957[1]. The 5-FU is one of the most commonly used chemotherapeutic agents in the treatment of solid cancers, including gastrointestinal, breast, head and neck and pancreatic[2]. It has been reported that its anticancer effect is due to the inhibition of RNA and protein synthesis, in addition to the inhibition of thymidylate synthase and deoxyribonucleic acid synthesis[3]. Although 5-FU continues to be the cornerstone of some cancer chemotherapy, it is the second most common cardiotoxic chemotherapeutic drug that can result in death[2,4,5]. Inflammation has been implicated in the pathogenesis of 5-FU-induced cardiotoxicity, as has oxidative stress due to increased reactive oxygen radicals (ROS)[6]. It has been stated that a main cause of 5-FU-induced cardiotoxicity is decreased antioxidant capacity[7]. Studies in the literature have reported that 5-FU causes oxidative and inflammatory cardiac damage by increasing cardiac enzymes, tissue malondialdehyde (MDA) and Interleukin-6 (IL-6) levels, decreasing Glutathione (GSH) and total antioxidant capacities[4]. Elevation of intracellular Calcium (Ca^{2+}) levels is assumed to be an important step in the pathogenesis of oxidative damage[8]. To date, no studies have shown that calcium channel blockers (CCBs) are beneficial in 5-FU-induced cardiotoxicity. However, there are studies showing that 5-FU increases intracellular free Ca^{2+} ion concentrations[9]. Altogether, this information suggested that the combination of CCBs and antioxidants may be useful in the treatment of 5-FU-induced cardiotoxicity.

Current study investigated the protective effects of lacidipine, which is an L-type CCB, against 5-FU-induced cardiotoxicity. Lacidipine is one of the most commonly used antihypertensive drugs due to its high vascular selectivity and tolerability, has an important role in the treatment of life-threatening cardiovascular diseases and shows antiatherosclerotic, antibacterial and antioxidant properties[10]. It has also been shown to protect cells from oxidative stress and inflammatory damage[11].

Another drug we investigated for protective effects against 5-FU-induced cardiotoxicity was carvacrol. Carvacrol is a phenolic monoterpenoid produced by plants, including *Origanum vulgare* (Greek oregano, marjoram), *Origanum majorana* (marjoram), *Satureja hortensis* (summer thyme), *Thymus vulgaris* (thyme) and *Satureja montana* (winter thyme)[12]. Carvacrol has anti-inflammatory, antioxidant, antibacterial, antidiabetic, antifungal, antitumor, antimutagenic, analgesic, anti-hepatotoxic, cardioprotective and antiparasitic properties[12,13]. There are no studies in the literature that investigated the effects of lacidipine, carvacrol and their combination on 5-FU-induced cardiotoxicity in rats. Therefore, the aim of this study was to investigate the biochemical effects of lacidipine, carvacrol and their combination on 5-FU-induced cardiotoxicity in rats.

MATERIALS AND METHODS

Study area: This study was performed at Erzincan Binali Yıldırım University, Faculty of Medicine, Erzincan, Türkiye from January, 2023 to February, 2023.

Animals: A total of 30 albino male Wistar rats weighing 275-290 g were used in this study. All of the rats were obtained from Erzincan Binali Yıldırım University Medical Experimental Application and Research Center. Before the experiment, the rats were housed and fed in a suitable environment (22) in the laboratory, with 12 hrs of light and 12 hrs of darkness. Animal experiments were performed in accordance with national guidelines for the use and care of laboratory animals and were approved by the Local Animal Ethics Committee (Ethics Committee No.: 2022/12, dated December 29, 2022).

Chemicals: The thiopental sodium used in the experiments was supplied by IE Ulagay (Istanbul, Türkiye), carvacrol was supplied by Sigma Chemical Co. (St. Louis, Missouri, USA), lacidipine was supplied by GlaxoSmithKline Drugs Türkiye (Istanbul, Türkiye) and 5-fluorouracil was supplied by KoçakFarma (Istanbul, Türkiye).

Experimental groups: The rats used in the experiment were divided into five groups based on the treatment administered: A healthy control (HC) group, 5-FU alone (FU), lacidipine+5-FU (LFU), carvacrol+5-FU (CFU) and lacidipine+carvacrol+5-FU (LCFU).

Experimental procedure: In the LFU group (n = 6), lacidipine was administered orally to the stomach by gavage at a dose of 4 mg kg^{-1}. Carvacrol was injected intraperitoneally (ip) at a dose of 50 mg kg^{-1} in the CFU (n = 6) group. In the LCFU (n = 6) group, lacidipine (4 mg kg^{-1}) was administered orally and carvacrol (50 mg kg^{-1}) was administered by ip using the method just described. The HC (n = 6) and FU (n = 6) groups were given the same volume of saline (0.9% NaCl) as the solvent. As 1 hr after administration of drugs and solvent, each animal in the LFU, CFU, LCFU and FU groups was injected ip with 5-FU at a dose of 100 mg kg^{-1}. Lacidipine and

carvacrol were given to the animals once per day for 10 days, while 5-FU was administered once per day on the 1st, 3rd and 5th days, for a total of three doses. On day 10, after taking blood samples from the tail veins, animals were sacrificed using high-dose anesthesia (50 mg kg^{-1} thiopental sodium) and heart tissues were removed. The excised heart tissues were examined biochemically and the biochemical test results obtained from the HC, LFU, CFU and LCFU groups were evaluated by comparing them with the test results obtained from the FU group.

MDA, GSH, superoxide dismutase, catalase and protein determination: The tissue samples taken from the animals were washed with physiological saline and placed in Petri dishes. The tissues were ground into powder in the presence of liquid nitrogen and then homogenized. After the homogenates were centrifuged, the supernatants were used for analysis to determine MDA, GSH, superoxide dismutase (SOD), catalase (CAT) and protein levels. The SOD activities and the GSH and MDA levels were measured with a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kits for the experimental animals, with each assay performed according to the kit instructions (Product no. 706002, 703002 and 10009055, respectively, Cayman Chemical Company, Ann Arbor, Michigan, USA). The CAT activities were measured according to the method suggested by Goth[14]. Protein levels were determined spectrophotometrically at 595 nm according to the Bradford method[15].

IL-6 analysis: The weights of the samples were measured and cut them all to a uniform size. Following quick freezing with liquid nitrogen, these materials were homogenized in a mortar and pestle. After melting, all of the samples were kept between 2 and 8°C. In order to collect the supernatants, PBS (pH 7.4) was added, vortexed for 10 sec and then centrifuged for 20 min at 10,000 × g. A commercial ELISA kit was used to detect IL-6 (ng L^{-1}) levels (Eastbiopharm Co. Ltd., Hangzhou, Zhejiang, China).

Determination of troponin I and creatine kinase MB: Troponin I (TP-I) levels were measured in plasma obtained using blood samples taken from the tail veins of the animals, using the enzyme-linked fluorescent assay (ELFA) technique in the VIDAS TP I Ultra kit (BioMerieux, Marcy l'Etoile, France). Similarly, creatine kinase MB (CK-MB) analyses were performed using a Roche cobas c701 system (Roche Diagnostics, Basel, Switzerland).

Statistical analysis: The IBM SPSS Statistics for Windows, Version 22.0, release 2013 (IBM Corp., Armonk, New York, USA) was used for the statistical analysis. The data of the parameters in the groups are presented as Mean±Standard Deviation (SD). The existence of a statistically significant difference between the groups was evaluated with the one-way ANOVA test and the existence of a statistically significant difference between the two groups was evaluated with Tukey's analysis. For all tests, the statistical significance threshold was set at 0.5.

RESULTS

As seen in Table 1, the SOD and CAT activities and the GSH levels were significantly lower ($p<0.001$) and the MDA, IL-6, TP-I and CK-MB levels were significantly higher ($p<0.001$) in the FU group than in the HC, LFU, CFU, or LCFU groups (Fig. 1-5). While there was a statistically significant difference between the HC group and the LFU and CFU groups in terms of 5-FU, MDA, GSH, SOD, CAT, IL-6, CK-MB and TP-I, the difference between the HC and LCFU groups could only be detected in terms of GSH ($p = 0.09$). In addition, MDA, IL-6, TP-I and CK-MB levels were lower (<0.001) and GSH, SOD and CAT levels were significantly higher (<0.001) in the LCFU group than in the LFU and CFU groups.

Table 1: Biochemical findings in the groups

Parameters	HC group	FU group	LFU group	CFU group	LCFU group	p-value
MDA (nmol mg^{-1} protein)	3.29±0.14[a]	6.89±0.13[b]	5.18±0.14[a,b]	4.167±0.094[a,b]	3.42±0.12[a]	<0.001
GSH (nmol mg^{-1} protein)	8.56±0.15[a]	3.30±0.09[b]	4.81±0.11[a,b]	6.23±0.09[a]	8.33±0.10[a,c]	<0.001
SOD (u mg^{-1} protein)	9.19±0.24[a]	3.62±0.13[b]	4.94±0.09[a,b]	7.29±0.06[a,b]	8.99±0.26[a]	<0.001
CAT (u mg^{-1} protein)	7.78±0.14[a]	3.23±0.05[b]	4.93±0.23[a,b]	6.95±0.09[a,b]	7.59±0.12[a]	<0.001
IL-6 (ng L^{-1})	2.40±0.11[a]	6.49±0.24[b]	3.59±0.08[a,b]	4.95±0.09[a,b]	2.50±0.07[a]	<0.001
TP-I (µg L^{-1})	0.015±0.005[a]	0.243±0.029[b]	0.113±0.018[a,b]	0.167±0.019[a,b]	0.022±0.004[a]	<0.001
CK-MB (U L^{-1})	178.17±12.75[a]	307.83±10.23[b]	226.83±14.25[a,b]	257.50±6.60[a,b]	187.50±11.20[a]	<0.001

HC: Healthy control group, FU: 5-FU group, LFU: Lacidipine+5-FU group, CFU: Carvacrol+5-FU group, LCFU: Lacidipine+carvacrol+5-FU group, MDA: Malondialdehyde, GSH: Glutathione, SDO: Superoxide dismutase, CAT: Catalase, IL-6: Interleukin-6, TP-I: Troponin I, CK-MB: Creatine kinase MB, [a]Statistically significant difference of the groups in comparison to the FU group ($p<0.001$), [b]Statistically significant difference of the groups in comparison to the HC group ($p<0.001$) and [c]Statistically significant difference of the groups in comparison to the HC group ($p<0.05$)

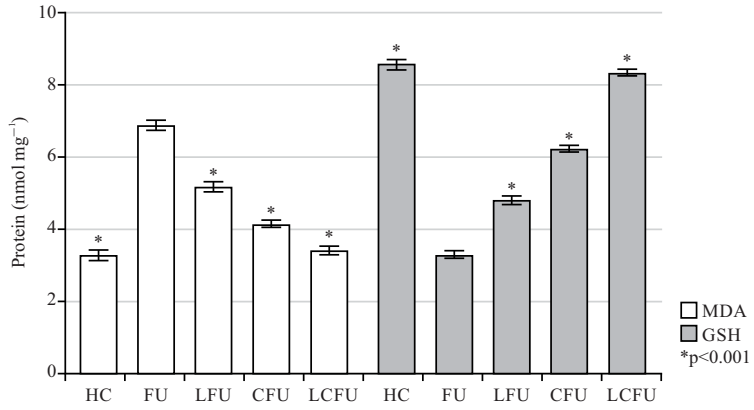

Fig. 1: MDA and GSH levels of the groups

HC: Healthy control group, FU: 5-FU group, LFU: Lacidipine+5-FU group, CFU: Carvacrol+5-FU group, LCFU: Lacidipine+carvacrol+5-FU group, MDA: Malondialdehyde, GSH: Glutathione and *Statistically significant difference of the groups in comparison to the FU group (p<0.001)

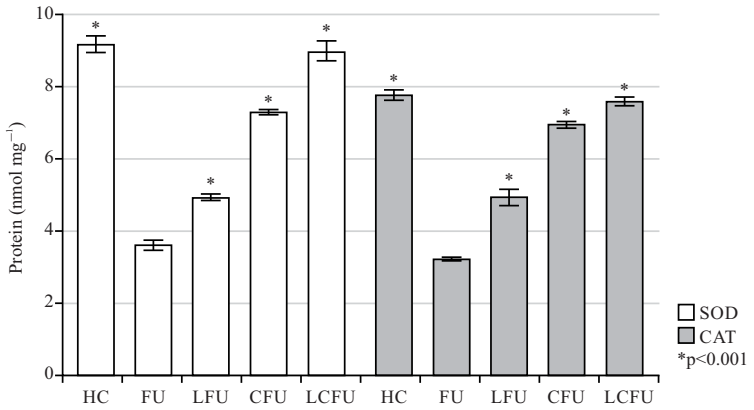

Fig. 2: SOD and CAT levels of the groups

HC: Healthy control group, FU: 5-FU group, LFU: Lacidipine+5-FU group, CFU: Carvacrol+5-FU group, LCFU: Lacidipine+carvacrol+5-FU group, SOD: Superoxide dismutase, CAT: Catalase and *Statistically significant difference of the groups in comparison to the FU group (p<0.001)

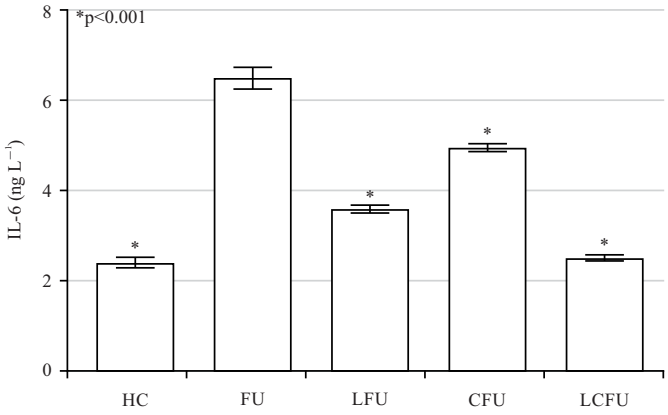

Fig. 3: IL-6 levels of the groups

HC: Healthy control group, FU: 5-FU group, LFU: lacidipine+5-FU group, CFU: carvacrol+5-FU group, LCFU: lacidipine+carvacrol+5-FU group, IL-6: interleukin-6 and *Statistically significant difference of the groups in comparison to the FU group (p<.001)

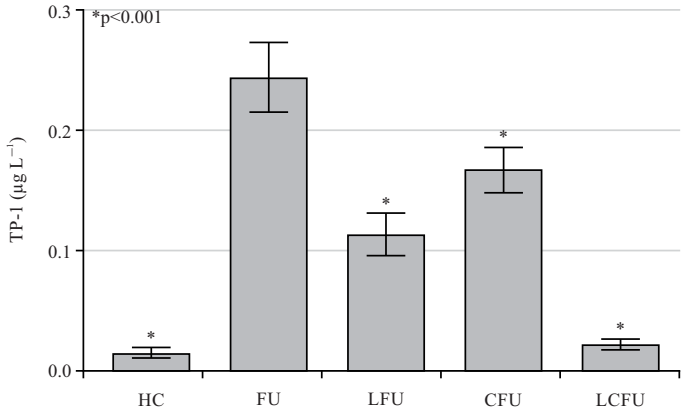

Fig. 4: TP-I levels of the groups

HC: Healthy control group, FU: 5-FU group, LFU: Lacidipine+5-FU group, CFU: Carvacrol+5-FU group, LCFU: Lacidipine+carvacrol+5-FU group, TP-I: Troponin I and *Statistically significant difference of the groups in comparison to the FU group (p<.001)

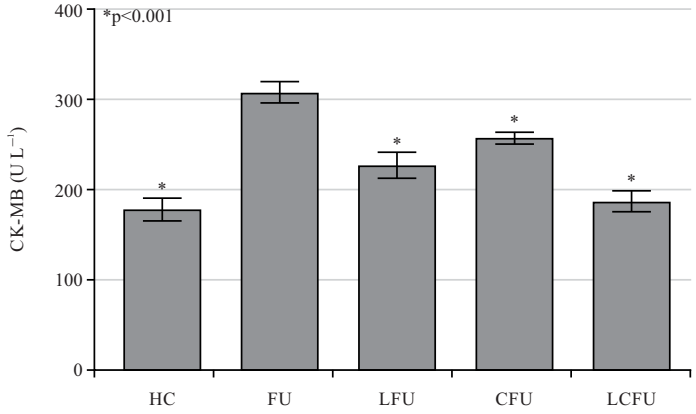

Fig. 5: CK-MB levels of the groups

HC: Healthy control group, FU: 5-FU group, LFU: Lacidipine+5-FU group, CFU: Carvacrol+5-FU group, LCFU: Lacidipine+carvacrol+5-FU group, CK-MB: Creatine kinase MB and *Statistically significant difference of the groups in comparison to the FU group (p<.001)

DISCUSSION

In this study, the protective effect of lacidipine, carvacrol and lacidipine-carvacrol combination against 5-FU-induced cardiotoxicity in rats was investigated biochemically. Current experimental results showed that 5-FU increased levels of MDA and IL-6 in the heart tissue of animals and decreased the levels of GSH, SOD and CAT. These findings revealed that 5-FU changes the oxidant/antioxidant balance in the heart tissue in favor of oxidants and triggers the proinflammatory mechanism.

Oxidative stress is defined as an imbalance resulting from an increase in oxidants and/or a decrease in antioxidants that can cause damage to biological systems. The underlying mechanism of oxidative stress has been shown to be excessive ROS production and antioxidant reduction[16]. Excessive increases in ROS production cause lipid peroxidation (LPO). Toxic secondary products such as MDA are formed during LPO. The MDA is one of the most popular and reliable markers of oxidative stress and its increase is associated with damage, while its decrease is associated with protection[17]. In this study, the increase in the amount of MDA in the heart tissue of animals treated with 5-FU was consistent with reports in the literature.

As seen from current experimental results, antioxidant parameter levels, including GSH, SOD and CAT, decreased in heart tissue with high MDA levels. It is known that GSH is a tripeptide compound consisting of glutamate, cysteine and

glycine. In summary, GSH is an antioxidant molecule that provides electrons for antioxidant enzymes and eliminates and scavenges ROS[18].

Another biomolecule that forms the first line of defense against free radicals is SOD. The SOD catalyzes the dismutation of the superoxide anion radical to hydrogen peroxide (H_2O_2)[19]. The oxidant H_2O_2 formed is then converted to water and oxygen (O_2) by CAT or glutathione peroxidase[20]. In the literature, there is data indicating that overproduced ROS in living tissues can be neutralized by endogenous GSH, SOD, CAT and other antioxidant defense systems. In the dysfunction of these defense systems, it is stated that oxidative stress may develop[16-20]. The findings in present study suggested that antioxidants are unable to neutralize ROS in untreated heart tissue and indicate that oxidative damage develops in heart tissue.

In current study, in addition to oxidative stress, a significant increase was observed in the production of IL-6 in heart tissue treated with 5-FU. This finding showed that 5-FU-induced cardiotoxicity triggers inflammation in the heart, which is consistent with the literature[21,22]. The IL-6 is a proinflammatory cytokine and its role in cardiovascular diseases is emphasized[23]. It is known that oxidative stress activates the transcription factor that regulates IL-6 expression[24]. It is assumed that IL-6 also participates in ROS production[25]. The present study experimental results and the information obtained from the literature suggested that 5-FU-induced cardiotoxicity causes inflammatory damage in heart tissue and that there is an important link between inflammatory damage and oxidative damage.

As can be understood from our experimental results, TP-I and CK-MB levels in blood serum increased in parallel with oxidative stress and inflammation markers. In the study by Attia *et al.*[26], it was reported that serum TP-I and CK-MB levels increased in parallel with the increase in oxidant levels in the heart tissue, in line with current study. In addition, it has been reported that increases in TP-I and CK-MB levels are also associated with an increase in IL-6 in heart tissue[27]. It has also been reported that the increases in serum TP-I and CK-MB levels are associated with increases in oxidant and intracellular Ca^{2+} [28]. This information indicates that CCBs may have positive effects in the 5-FU-induced cardiotoxicity model.

In this study experimental results, it was determined that lacidipine prevents the increase of MDA and the decrease of enzymatic antioxidant levels, such as GSH, SOD and CAT, in cardiotoxicity related to 5-FU in the heart tissue. No studies were found in the literature investigating the effect of lacidipine on oxidative heart damage due to 5-FU-induced cardiotoxicity. However, in a reported study, lacidipine significantly reduced mitochondrial ROS production, NADPH activity and MDA levels induced by H_2O_2 in cultured endothelial cells[29]. However, in another study, lacidipine demonstrated its antioxidant activity by preventing the increase of MDA and the decrease of antioxidants SOD and CAT in heart tissue[30]. Antioxidant effects of CCBs are observed especially in CCBs with highly lipophilic chemical structures (agents that quench free radical reaction by facilitating proton donation and resonance stabilization mechanisms)[31,32]. The CCBs such as amlodipine and lacidipine can reduce peroxide accumulation by donating protons to lipid peroxide molecules when placed near polyunsaturated fatty acids in the membrane[32]. In this study, the increase of IL-6, which we consider to be a proinflammatory molecule, was also prevented by lacidipine. The relationship between oxidative stress and inflammation is known and oxidative stress activates IL-6 expression, while IL-6 induces ROS production[24,25]. It has also been stated that IL-6 expression is associated with intracellular calcium levels[33]. This information obtained from the literature supports our experimental results that lacidipine inhibited IL-6 increases in heart tissue.

Carvacrol, the other agent whose effect was evaluated in our study, also inhibited the increase in MDA levels and the decrease in GSH, SOD and CAT levels due to 5-FU administration in heart tissue. Carvacrol has strong antioxidant properties and may be effective in the treatment and prevention of many diseases based on oxidative stress[12]. The antioxidant activity of carvacrol is supposed to formed by the hydroxyl group in the aromatic ring[34]. In a study conducted in aged rats, *T. vulgaris* reduced the age-related decrease in SOD enzymes in liver and heart tissues[35]. Similarly, carvacrol showed positive effects on antioxidant capacity in heart tissue. In the study from Karakurt *et al.*[27], carvacrol was shown to alleviate heart damage in rats by antagonizing the effects of ketamine on MDA, SOD and GSH. In another study by Chen *et al.*[36], it was reported that administration of carvacrol significantly protected heart function, reduced the size of myocardial infarction, increased SOD and CAT levels and decreased MDA levels in cardiac ischemia-reperfusion injury. The anti-inflammatory activity of carvacrol is known. Carvacrol has been reported to exert its anti-inflammatory effects by inhibiting inflammatory cytokine levels and expression of inducible nitric oxide synthase and cyclooxygenase-2[37]. It was reported by Gatica *et al.*[38] that carvacrol has the ability to inhibit the increase of IL-6 and other proinflammatory cytokines. Carvacrol also attenuated the increase of IL-6 in heart tissue in ketamine-related cardiotoxicity[27]. It is known that there is a positive correlation between the increase in intracellular Ca^{2+} concentration and the increase in ROS and

IL-6 production[33]. In addition, the expression that carvacrol has the ability to block Ca^{2+} channels revealed that current experimental results were in agreement with the literature[39]. Current results suggested that oxidative stress and inflammation play an important role in the pathogenesis of 5-FU-induced cardiotoxicity. While signs of oxidative damage and inflammatory heart damage were observed following 5-FU administration, lacidipine and carvacrol almost equally prevented this oxidative and inflammatory heart damage.

CONCLUSION

The combination of lacidipine and carvacrol reduced heart damage better than either lacidipine or carvacrol alone. This study experimental results showed that lacidipine and carvacrol are effective in 5-FU-induced cardiotoxicity, but these two agents together provide a more effective treatment.

SIGNIFICANCE STATEMENT

The 5-FU is one of the most commonly used cardiotoxic chemotherapeutic agents. The literature states that inflammation and oxidative stress play a role in the etiopathogenesis of 5-FU-induced cardiotoxicity. The current study suggests that lacidipine and carvacrol, each with antioxidant and anti-inflammatory properties, have a potent protective effect against 5-FU-induced cardiotoxicity when used in combination.

REFERENCES

1. Polk, A., M. Vaage-Nilsen, K. Vistisen and D.L. Nielsen, 2013. Cardiotoxicity in cancer patients treated with 5-fluorouracil or capecitabine: A systematic review of incidence, manifestations and predisposing factors. Cancer Treat. Rev., 39: 974-984.
2. Polk, A., K. Vistisen, M. Vaage-Nilsen and D.L. Nielsen, 2014. A systematic review of the pathophysiology of 5-fluorouracil-induced cardiotoxicity. BMC Pharmacol. Toxicol., Vol. 15. 10.1186/2050-6511-15-47.
3. Longley, D.B., D.P. Harkin and P.G. Johnston, 2003. 5-fluorouracil: Mechanisms of action and clinical strategies. Nat. Rev. Cancer, 3: 330-338.
4. Refaie, M.M.M., S.A. Abdel-Gaber, S.A.A. El Rahman, S.M.N. Abdel Hafez and H.M. Khalaf, 2022. Cardioprotective effects of bosentan in 5-fluorouracil-induced cardiotoxicity. Toxicology, Vol. 465. 10.1016/j.tox.2021.153042.
5. Sara, J.D., J. Kaur, R. Khodadadi, M. Rehman and R. Lobo *et al*., 2018. 5-fluorouracil and cardiotoxicity: A review. Ther. Adv. Med. Oncol., Vol. 10. 10.1177/1758835918780140.
6. Lamberti, M., S. Porto, M. Marra, S. Zappavigna and A. Grimaldi *et al*., 2012. 5-Fluorouracil induces apoptosis in rat cardiocytes through intracellular oxidative stress. J. Exp. Clin. Cancer Res., Vol. 31. 10.1186/1756-9966-31-60.
7. Durak, L., M. Karaayvaz, M. Kavutcu, M.Y.B. Cimen, M. Kacmaz, S. Buyukkocak and H.S. Ozturk, 2000. Reduced antioxidant Defense capacity in myocardial tissue from guinea pigs treated with 5-fluorouracil. J. Toxicol. Environ. Health Part A, 59: 585-589.
8. Kalogeris, T., C.P. Baines, M. Krenz and R.J. Korthuis, 2012. Cell Biology of Ischemia/Reperfusion Injury. In: International Review of Cell and Molecular Biology, Jeon, K.W. (Ed.), Academic Press, Cambridge, Massachusetts, ISBN: 9780123943095, pp: 229-317.
9. Deveci, H.A., M. Nazıroğlu and G. Nur, 2018. 5-Fluorouracil-induced mitochondrial oxidative cytotoxicity and apoptosis are increased in MCF-7 human breast cancer cells by TRPV1 channel activation but not *Hypericum perforatum* treatment. Mol. Cell. Biochem., 439: 189-198.
10. Chebrolu, T.S., L. Kumar and R. Verma, 2021. Lacidipine: Review of analytical methods developed for pharmaceutical dosage forms and biological fluids. Bioanalysis, 13: 1011-1024.
11. Suleyman, B., Z. Halici, F. Odabasoglu and F. Gocer, 2012. The effect of lacidipine on indomethacin induced ulcers in rats. Int. J. Pharmacol., 8: 115-121.
12. Baser, K.H.C., 2008. Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils. Curr. Pharm. Des., 14: 3106-3119.
13. Dhama, K., R. Tiwari, S. Chakraborty, M. Saminathan and A. Kumar *et al*., 2014. Evidence based antibacterial potentials of medicinal plants and herbs countering bacterial pathogens especially in the era of emerging drug resistance: An integrated update. Int. J. Pharmacol., 10: 1-43.
14. Goth, L., 1991. A simple method for determination of serum catalase activity and revision of reference range. Clin. Chim. Acta, 196: 143-151.
15. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.
16. Forman, H.J. and H. Zhang, 2021. Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. Nat. Rev. Drug. Discovery, 20: 689-709.
17. Ayala, A., M.F. Muñoz and S. Argüelles, 2014. Lipid peroxidation: Production, metabolism and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid. Med. Cell. Longevity, Vol. 2014. 10.1155/2014/360438.
18. Liu, T., L. Sun, Y. Zhang, Y. Wang and J. Zheng, 2022. Imbalanced GSH/ROS and sequential cell death. J. Biochem. Mol. Toxicol., Vol. 36. 10.1002/jbt.22942.
19. Yan, Z. and H.R. Spaulding, 2020. Extracellular superoxide dismutase, a molecular transducer of health benefits of exercise. Redox Biol., Vol. 32. 10.1016/j.redox.2020.101508.

20. Jelic, M.D., A.D. Mandic, S.M. Maricic and B.U. Srdjenovic, 2021. Oxidative stress and its role in cancer. J. Cancer Res. Ther., 17: 22-28.
21. Okamoto, M., H. Kasetani, R. Kaji, H. Goda and G. Ohe *et al*., 1998. *cis*-Diamminedichloroplatinum and 5-fluorouracil are potent inducers of the cytokines and natural killer cell activity *in vivo* and *in vitro*. Cancer Immunol. Immunother., 47: 233-241.
22. Arafah, A., M.U. Rehman, A. Ahmad, K.M. AlKharfy, S. Alqahtani, B.L. Jan and N.M. Almatroudi, 2022. Myricetin (3,3 ,4 ,5,5 ,7-hexahydroxyflavone) prevents 5-fluorouracil-induced cardiotoxicity. ACS Omega, 7: 4514-4524.
23. Ridker, P.M. and M. Rane, 2021. Interleukin-6 signaling and anti-interleukin-6 therapeutics in cardiovascular disease. Circ. Res., 128: 1728-1746.
24. Kumari, N., B.S. Dwarakanath, A. Das and A.N. Bhatt, 2016. Role of interleukin-6 in cancer progression and therapeutic resistance. Tumor. Biol., 37: 11553-11572.
25. Han, F., S. Li, Y. Yang and Z. Bai, 2021. Interleukin-6 promotes ferroptosis in bronchial epithelial cells by inducing reactive oxygen species-dependent lipid peroxidation and disrupting iron homeostasis. Bioengineered, 12: 5279-5288.
26. Attia, A.A., J.M. Sorour, N.A. Mohamed, T.T. Mansour, R.A. Al-Eisa and N.S. El-Shenawy, 2023. Biochemical, histological, and ultrastructural studies of the protective role of vitamin E on cyclophosphamide-induced cardiotoxicity in male rats. Biomedicines, Vol. 11. 10.3390/biomedicines11020390.
27. Karakurt, T.C.Ö., İ. Emir, Z. Bedir, K.T.O. Erdem, H. Süleyman, C. Sarıgül and A.S. Mendil, 2022. Effects of carvacrol on ketamine-induced cardiac injury in rats: An experimental study. Drug Chem. Toxicol., 10.1080/01480545.2022.2155664.
28. Gomaa, A.M.S., A.T. Abdelhafez and H.A. Aamer, 2018. Garlic (*Allium sativum*) exhibits a cardioprotective effect in experimental chronic renal failure rat model by reducing oxidative stress and controlling cardiac Na^+/K^+-ATPase activity and Ca^{2+} levels. Cell Stress Chaperones, 23: 913-920.
29. Liu, X., Z. Huang, Y. Zhang, X. Shui, F. Liu, Z. Wu and S. Xu, 2021. Lacidipine ameliorates the endothelial senescence and inflammatory injury through CXCR7/P38/C/EBP-β signaling pathway. Front. Cardiovasc. Med., Vol. 8. 10.3389/fcvm.2021.692540.
30. Bayir, Y., Z. Halici, E. Karakus, A. Albayrak and I. Ferah *et al*., 2012. Comparing effects of lacidipine, ramipril, and valsartan against experimentally induced myocardial infarcted rats. Cardiovasc. Toxicol., 12: 166-174.
31. Mason, R.P., M.F. Walter, M.W. Trumbore, E.G. Olmstead Jr and P.E. Mason, 1999. Membrane antioxidant effects of the charged dihydropyridine calcium antagonist amlodipine. J. Mol. Cell. Cardiol., 31: 275-281.
32. Godfraind, T. and S. Salomone, 2015. Ambiguities in dietary antioxidant supplementation compared to calcium channel blockers therapy. Front. Pharmacol., Vol. 6. 10.3389/fphar.2015.00010.
33. Didion, S.P., 2017. Cellular and oxidative mechanisms associated with interleukin-6 signaling in the vasculature. Int. J. Mol. Sci., Vol. 18. 10.3390/ijms18122563.
34. Guimaraes, A.G., G.F. Oliveira, M.S. Melo, S.C.H. Cavalcanti and A.R. Antoniolli *et al*., 2010. Bioassay-guided evaluation of antioxidant and antinociceptive activities of carvacrol. Basic. Clin. Pharmacol. Toxicol., 107: 949-957.
35. Youdim, K.A. and S.G. Deans, 2000. Effect of thyme oil and thymol dietary supplementation on the antioxidant status and fatty acid composition of the ageing rat brain. Br. J. Nutr., 83: 87-93.
36. Chen, Y., L. Ba, W. Huang, Y. Liu and H. Pan *et al*., 2017. Role of carvacrol in cardioprotection against myocardial ischemia/reperfusion injury in rats through activation of MAPK/ERK and Akt/eNOS signaling pathways. Eur. J. Pharmacol., 796: 90-100.
37. Landa, P., L. Kokoska, M. Pribylova, T. Vanek and P. Marsik, 2009. *In vitro* anti-inflammatory activity of carvacrol: Inhibitory effect on COX-2 catalyzed prostaglandin E_2 biosynthesisb. Arch. Pharm. Res., 32: 75-78.
38. Gatica, S., F. Eltit, J.F. Santibanez, D. Varela, C. Cabello-Verrugio and F. Simon, 2019. Expression suppression and activity inhibition of TRPM7 regulate cytokine production and multiple organ dysfunction syndrome during endotoxemia: A new target for sepsis. Curr. Mol. Med., 19: 547-559.
39. Dantas, B.P.V., Q.L. Alves, K.S. de Assis, T.P. Ribeiro and M.M. de Almeida *et al*., 2015. Participation of the TRP channel in the cardiovascular effects induced by carvacrol in normotensive rat. Vasc. Pharmacol., 67-69: 48-58.