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ORIGINAL ARTICLE

A Combination of Virgin Coconut Oil and Extra Virgin Olive Oil Elicits Superior Protection Against Doxorubicin Cardiotoxicity in Rats

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ABSTRACT

Objectives: The use of the chemotherapy agent doxorubicin (DOX) is associated with free radical formation that may lead to cardiotoxicity. Virgin coconut oil (VCO) and extra virgin olive oil (EVOO) are plant-based oil that is rich in antioxidants. This study examined the protective effects of VCO and EVOO combination to reduce DOX acute cardiotoxicity in rats.

Materials and Methods: Twenty-five male rats (180-200 g) were divided into the following groups: Group I as a control, group II was given DOX *i.p.* injection of 25 mg/kg body weight (b.w.), group III to V received peroral administration of either VCO, EVOO or VCO-EVOO (1:1) combination at a dose of 10 mL/kg b.w. for 6 days before receiving DOX *i.p.* injection. After 24 hours from DOX injection, blood samples and organs were collected. Cardiac biomarkers, such as serum glutamic-oxaloacetic transaminase (SGOT), lactate dehydrogenase (LDH), and creatine kinase-MB (CKMB) were analyzed followed by histopathological examination.

Results: The administration of EVOO alone was found to reduce the marked elevation of SGOT, LDH, and CKMB levels in DOX-treated rats ($p<0.05$), while VCO administration only significantly reduced LDH and CKMB levels. However, when both oils were used in combination, the protective effect was shown to be more powerful since all cardiac biomarker levels were maintained at near-normal levels ($p<0.05$). Histopathological analysis showed a significant improvement in the myocardial tissue structures after pre-treatment with VCO-EVOO combination.

Conclusion: The administration of VCO and EVOO in combination was superior to elicit protection against DOX-induced cardiotoxicity compared to their individual application in rats.

Key words: Doxorubicin, cardiac toxicity, virgin coconut oil, extra virgin olive oil

INTRODUCTION

Doxorubicin (DOX) is an anthracycline isolated from *Streptomyces peucetius* var. *caesius* in the 1970s.[1] DOX has been widely used for treating various malignancies, including breast and lung cancers, lymphomas, myeloma, and sarcomas.[2] However, clinical studies have reported approximately 3-33% of patients, who received DOX developed subclinical cardiomyopathy and some progress to congestive heart failure, even with an average cumulative dose of 300 mg/m^2.[3,4]

The mechanism of DOX toxicity is mediated by the metabolic conversion of DOX to its secondary alcohol doxorubicinol.[5] This metabolite then interacts with iron and initiates the formation of reactive oxygen species (ROS) that subsequently damage cellular macromolecules.[6] DOX can also form semiquinone radical intermediates that react with oxygen to produce superoxide anion radicals, which in turn produce hydrogen peroxide and hydroxyl radicals that mostly attack lipids, proteins, and DNA molecules.[7] As a result, DOX-treated patients are at high risks of experiencing cardiotoxicity.

Bioactive compounds that can protect the heart against cardiotoxicity have been continuously pursued over the past few decades. Virgin coconut oil (VCO) and extra virgin olive oil (EVOO) are two different plant-based oils that provide health benefits to cardiac tissue and function. VCO contains

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antioxidants mostly in the form of polyphenols, which have the capability to inhibit low-density lipoprotein (LDL) oxidation in rats.[8] In separate studies, VCO or EVOO administration increases antioxidant enzyme activities and improve the body's defense system against oxidative stress.[9,10] Moreover, the hydroxytyrosol and oleuropein content of EVOO can act as free radical scavengers and inhibit the oxidation of LDL.[11] Accordingly, this study compared the cardioprotective effects of VCO, EVOO, and their combination (1:1) on DOX-induced cardiotoxicity in rats.

MATERIALS AND METHODS

Materials

VCO (Avcol®, Indonesia) and EVOO (CV. Asy Syifa, Indonesia) used in this study are registered in the Indonesian Food and Drug Administration and were purchased from a registered pharmacy. DOX HCl 50 mg/25 mL (Kalbe Farma, Indonesia) and sodium chloride (NaCl) 0.9% were obtained from a local hospital.

Animals

Male Wistar rats weighed at 180-200 g (n= 25) were procured from a laboratory animal breeder in Makassar, Indonesia. The animals were cared for in a laboratory with a 12 hour dark/light cycle and provided with food and drink daily. The animals were acclimatized to laboratory environmental conditions for 14 days before the experiment was carried out. All animal protocols were performed based on institutional guidelines for animal laboratory handling and registered under an institutional ethical clearance number of UH21020059 from the Ministry of National Education, University of Hasanuddin Faculty of Medicine, Health Research Ethics Committee.

Experimental design

A simplified scheme of the experimental protocol is illustrated in Figure 1. The rats were divided into 5 groups where each group consisted of 5 rats. Rats' blood samples (3 mL) were withdrawn before treatment initiation to provide serum biomarker baseline levels. Group I as a control group was intraperitoneally (*i.p.*) injected with 0.9% NaCl solution as a placebo, while group II was *i.p.* injected with 25 mg/kg b.w. of DOX on the 7th day of the experiment. Group III to V received pre-treatments of either VCO (10 mL/kg), EVOO (10 mL/kg), or VCO-EVOO (1:1 at 10 mL/kg dose), respectively. The oil treatment was administered using an oral cannula for 6 days, before receiving DOX *i.p.* injection on the next day. After 24 h from DOX injection, rats were anesthetized with diethyl ether, and 3 mL of blood samples were obtained from lateral veins using vacutainer tubes containing ethylenediaminetetraacetic acid. Blood samples were centrifuged (Hettich®) at a speed of 2000 rpm for 25 min. The serum was collected immediately and stored at -20°C until biomarker analysis was performed. The experimental protocols used in this study was based on Djabir et al.[12] study which also delivered a short-term pre-treatment to protect against cardiotoxicity induced by a single *i.p.* injection of DOX (25 mg/kg). A short-term pre-treatment with VCO (10 mL/kg/day) for 7 days was shown to significantly reduce paracetamol-induced hepatotoxicity.[13] Meanwhile, pre-treatments with EVOO phytochemicals, such as oleuropein or hydroxytyrosol, for up to 7 days sufficiently elicit protection against a range of drug-induced cardiotoxicities.[14-16]

Biomarker analysis

The levels of serum glutamic oxaloacetic transaminase (SGOT), lactate dehydrogenase (LDH), and plasma biomarker creatine kinase-MB (CKMB) were analyzed using diagnostic

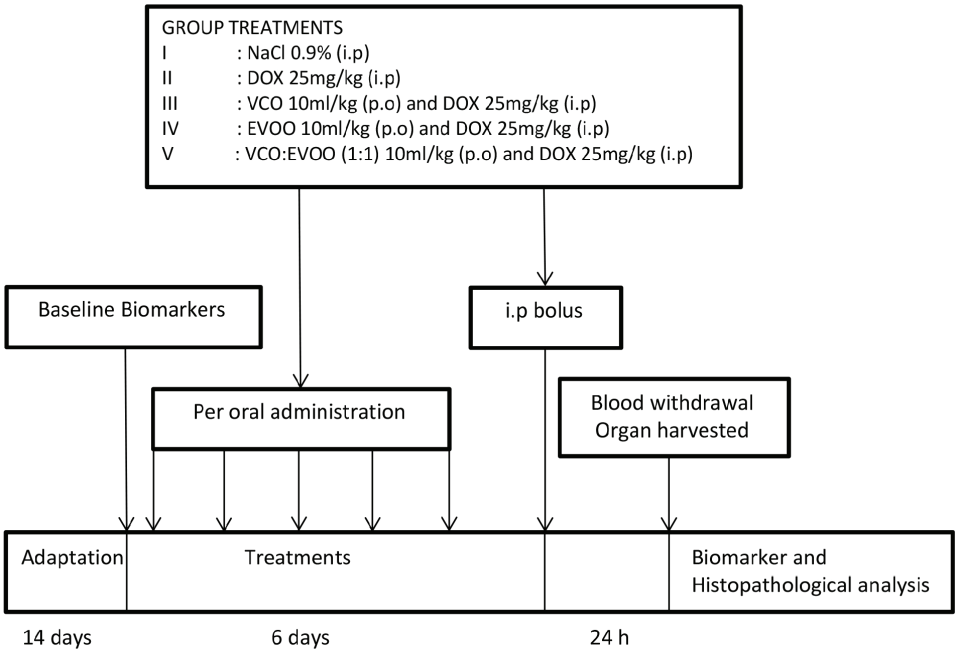

Figure 1. The scheme of experimental protocols

kits obtained from Human Diagnostic World (Germany). All analytical procedures were carried out according to the kits' instructions. The absorbance was measured using Humalyzer 3500 (Human Diagnostic World) instrumentation.

Histopathological examination

Following a blood sample collection, rats were euthanized using a cervical dislocation method. The heart was carefully removed and fixated in 10% formalin in phosphate buffer saline and embedded in paraffin blocks. Sections of 5 µm thick were serially sliced with a microtome and stained with hematoxylin and eosin. The microscopic observation of the longitudinal section of the heart tissue was performed by a veterinary pathologist, especially in the area of the ventricles, using a light microscope (Olympus®) at 40X magnification.

Statistical analysis

The numerical data were presented as mean ± standard error of the mean. The distribution of data normality was tested using a Shapiro-Wilk normal distribution analysis. Normally distributed data were then analyzed with One-Way ANOVA followed by a *post-hoc* Tukey's honestly significant difference test. Statistical analysis was declared significant if the *p* value was below 0.05 ($p<0.05$).

RESULTS

Biomarker analysis

The result of cardiac biomarker analysis is illustrated in Figure 2. The healthy control did not show significant changes in the CKMB, LDH, and SGOT levels after receiving 0.9% NaCl injection as a placebo (Figure 2). However, in the DOX group, marked increases in CKMB, LDH, and SGOT levels were experienced in all rats. The CKMB, LDH, and SGOT levels of the DOX group were at least three times their baseline values, and this biomarker upsurge was detected as soon as 24 hours from DOX *i.p.* injection at a dose of 25 mg/kg b.w. ($p<0.01$).

Apart from the DOX group, the VCO treatment group also experienced an increase in SGOT value after receiving DOX injection, which was significantly higher than the control group ($p<0.01$). The other cardiac biomarkers, such as LDH and CKMB levels, also rose about 50% above the normal control; nevertheless, the statistical analysis did not reach a significant difference compared to the control group.

The administration of EVOO at 10 mL/kg resulted in a near-normal level of CKMB similar to the control group (298 ± 33 *vs.* 214 ± 40 mg/dL). However, the LDH level of the EVOO group increased twice as much as that of the normal controls (597 ± 98 *vs* 218 ± 98 mg/dL); yet, it was found not statistically significant. The SGOT level also significantly increased in the EVOO group, but it was still significantly lower than that of the DOX-treated animals ($p<0.05$). In contrast, the administration of VCO-EVOO combination prevented the increase in CKMB and LDH, resulting in normal biomarker levels. Even though the elevation of SGOT level was still experienced by the VCO-EVOO group, it was significantly attenuated compared to the DOX group ($p<0.05$).

Histopathological examination

The normal control that was not subjected to the DOX injection showed regular cardiac myocyte shapes and structures (Figure 3A1). The bands and nucleus of cardiac myocytes and the myofibrils were clearly clear. There were barely inflammatory cells or necrotic damage found in the area of myocytes. In contrast, the DOX group experienced mild-to-moderate histopathological injuries. Histopathological changes in the heart muscle cells were evident and profound in the area of myocytes. Moderate damage was observed in most DOX-treated rats, which was characterized by hyper-eosinophilic cytoplasm and necrotic cell nuclei, myocardial cell atrophy, loss of nuclei, myolysis, infiltration of inflammatory cells, and hemorrhagic area (Figure 3B1-B3).

Figure 4 shows the representative microscopic images of cardiomyocyte histopathological changes found in rats treated with VCO, EVOO, or their combination. In the VCO-treated rats (Figure 4C1, C2), most cardiac sections showed necrotic cells

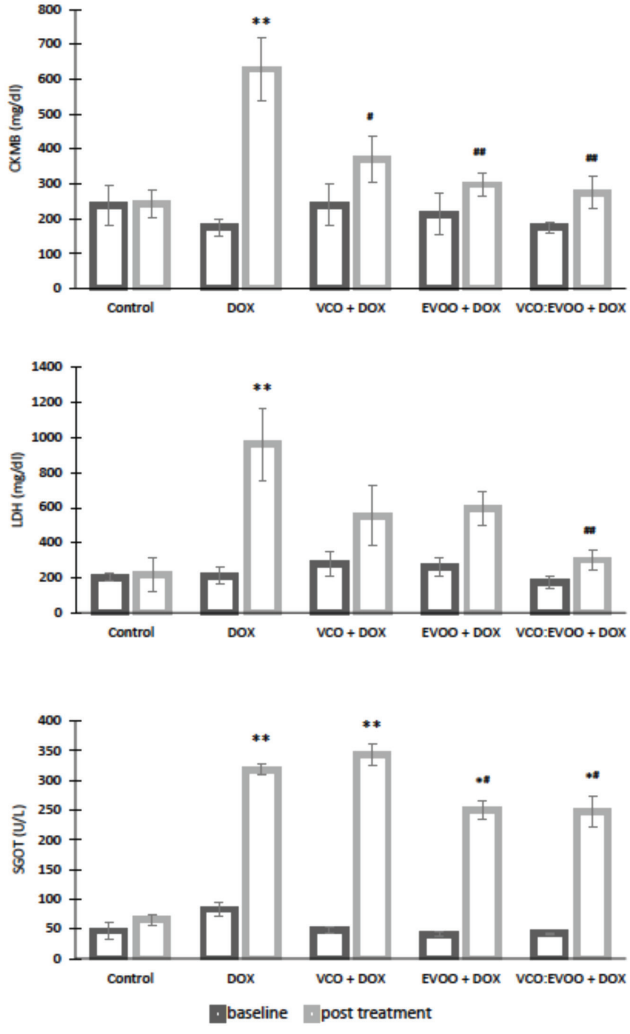

Figure 2. Comparison of creatine kinase-MB, lactate dehydrogenase, and serum glutamic-oxaloacetic transaminase levels between treatment groups at baseline and post-treatment

DOX: Doxorubicin, VCO: Virgin coconut oil, EVOO: Extra virgin olive oil

*p=0.05 compared to control. p=0.05 compared to DOX group

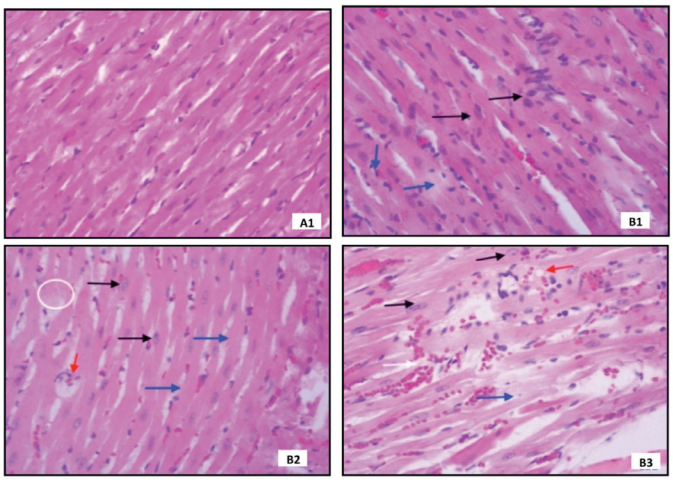

Figure 3. Representative of microscopic images of cardiac tissues in the control and DOX-treated rats. The control (A1) showed normal architecture of myocytes. cells with a normal structure with a magnification of 40X. The DOX group (B1, B2, and B3) showed necrotic cells (black arrow), myocardial muscle atrophy (blue arrow), vacuolar degeneration and hemorrhage (red arrow), and myolysis (white)

DOX: Doxorubicin

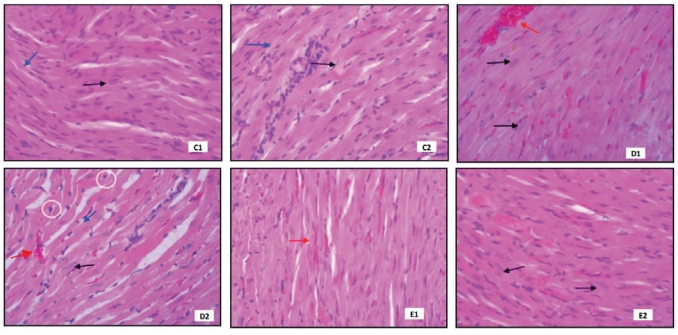

Figure 4. Representative of microscopic images of cardiac tissues in DOX treated rats that received pre-treatment with VCO (C1-2), EVOO (D1-2), and VCO-EVOO (E1-2). Necrotic myocardial cells (black arrow), muscle atrophy (blue arrow), inflammatory cells (white circle), and hemorrhagic area (red arrow)

DOX: Doxorubicin, VCO: Virgin coconut oil, EVOO: Extra virgin olive oil

and cardiomyocyte atrophy. The degree of myocardial injury was found mild to moderate (Table 1). With EVOO pre-treatment, the injection of DOX still resulted in mild-to-moderate damage, shown by the presence of necrotic cells, atrophy of cardiomyocytes, and inflammatory cells in cardiac tissue of rats (Figure 4D1, D2). Conversely, the administration of VCO and EVOO combination could reduce the presence of myocardial pathological damage. In this group, some histopathological changes were found, including hemorrhage and necrotic cells, but the degree was minimal (Figure 4E1, E2). Only two of five animals in this group had minimal myocardial damage, while the three others did not elicit myocardial injury similar to that observed in the normal controls (Table 1).

DISCUSSION

In spite of its chemotherapy benefit, the use of the DOX regimen is closely related to increased incidence of cardiotoxicity in

Table 1. Histopathological changes and scores found in each animal in the treatment groups

Groups	Rat	Histopathological changes (score)
Control	A1	None (0)
	A2	None (0)
	A3	None (0)
	A4	None (0)
	A5	None (0)
DOX	B1	Necrotic cells and atrophy of cardiomyocytes (2)
	B2	Necrotic cells, vacuolization, atrophy of cardiomyocytes, and myolysis (3)
	B3	Necrotic cells, atrophy of cardiomyocytes, vacuolar degeneration, and hemorrhage (3)
	B4	Necrotic cells, atrophy of cardiomyocytes and inflammatory cells (2)
	B5	Necrotic cells and atrophy of cardiomyocytes (2)
VCO + DOX	C1	Necrotic cells and atrophy of cardiomyocytes (2)
	C2	Necrotic cells and atrophy of cardiomyocytes (2)
	C3	Necrotic myocardial cells (1)
	C4	Cardiomyocyte degeneration and inflammatory cells (1)
	C5	Cardiomyocyte degeneration (1)
EVOO + DOX	D1	Cardiomyocyte degeneration and hemorrhage (1)
	D2	Necrotic cells, atrophy of cardiomyocytes and inflammatory cells (2)
	D3	Necrotic cells and atrophy of cardiomyocytes (2)
	D4	Cardiomyocyte degeneration and hemorrhage (1)
	D5	Necrotic cells, atrophy of cardiomyocytes and inflammatory cells (1)
VCO: EVOO + DOX	E1	Mild hemorrhage (1)
	E2	Degenerative cells (1)
	E3	None (0)
	E4	None (0)
	E5	None (0)

The level of damage was none to minimal (0), mild (1), moderate (2), and intense (3). DOX: Doxorubicin, VCO: Virgin coconut oil, EVOO: Extra virgin olive oil

cancer patients.[17] Consequently, various research is imposed in the pursuit of cardioprotective agents that can impede DOX-induced toxicity, including olive oil,[18] taurine,[19] vitamin E,[20] and vitamin C.[21,22] The progression of DOX cardiotoxicity is instigated by the formation of free radicals in the metabolic process of the chemotherapy agent, leading to impaired calcium transport in the sarcolemma, increased production of lipid peroxides, and the release of TNF-, interleukin-2, and free cytokines.[23] In this study, the administration of DOX was employed to trigger cardiotoxicity in rats. A previous study has shown that DOX *i.p.* injection (25 mg/kg) in rats was associated with increased ROS formation, myocyte necrosis, cardiomyocyte atrophy, vacuolar degeneration, and hemorrhage.[12]

In this present study, a single injection of DOX (25 mg/kg) was shown to trigger a significant elevation of cardiac injury biomarkers, demonstrating its deleterious effects on cardiac cells. Additionally, the toxic effect of DOX manifests in histopathological lesions, mostly portrayed by the presence of necrosis and atrophy of cardiomyocytes. The pre-treatment with either EVOO or VCO-EVOO combination in DOX-treated rats caused an attenuation of SGOT, LDH, and CKMB elevations. However, we found that the combination of VCO and EVOO was more effective to restore cardiac biomarkers to normal levels compared to the administration of EVOO as single preparation. Interestingly, in this study, although VCO could ease the increase of CKMB and LDH levels, it failed to improve the SGOT level in DOX-treated rats. A similar result was also depicted in the histopathological examination. It is shown that DOX-induced histological alteration was mostly improved with VCO and EVOO combination compared to VCO or EVOO alone. Indeed, the application of the oil combination before DOX injection apparently capable of preserving the normal features of cardiac muscle cells.

The health benefit of EVOO consumption has long been recognized due to the high concentration of monounsaturated fatty acids (MUFAs) content, especially oleic acid. Oleic acid increases plasma high density lipoprotein and reduce LDL cholesterol levels.[24] For this reason, oleic acid is expected to prevent cardiovascular disease, which is the leading cause of death in industrialized countries. Previously, several studies have shown the cardioprotective effect of olive oil on DOX-induced cardiotoxicity.[18,25] This effect was bestowed by the antioxidative compounds contained in olive oil,[10] including oleuropein and hydroxytyrosol.[11,26] Instead of hindering anticancer activity of DOX, the antioxidant activity of EVOO was found to synergistically improve DOX effect as chemotherapy.[27]

VCO has also been known to provide benefits in eliminating stress, weight loss, lowering cholesterol and LDL levels, maintaining blood pressures, circulatory disorders, as an immunomodulator and anti-inflammatory agent.[28,29] Indeed, VCO's benefits on endogenous antioxidant and defense mechanism have been known for years.[30,31] These promising effects have placed VCO as one of the virtuous candidates for cardioprotective agents. Nonetheless, apart from studies focusing on VCO's hepatoprotective, antioxidant, and immunomodulatory effects,[32-34] there is lack of studies that focus on VCO's cardioprotection against DOX toxicity.

In our study, it is demonstrated that VCO's cardioprotection against DOX toxicity was inferior compared to EVOO. Various studies have shown that the health benefits of EVOO are far beyond MUFA effects since EVOO is also rich in phenolic contents, tocopherol, squalene, phytosterols, triterpenoids, and β-carotene.[35] Compared to coconut oil, olive oil was found to be superior in reducing atherosclerotic plaque in hamsters fed with a high-fat diet.[36] Another study has also confirmed this result by showing better prevention of hepatic steatosis, insulin sensitivity, inflammation, and fatty acid oxidation in mice treated with olive oil compared to coconut oil.[37] Having said that the important finding in this present study is that EVOO's protective effects were augmented when it was combined with VCO. The combination could significantly improve cardiac injury biomarkers as well as histological features of cardiac tissues compared to EVOO alone. It is believed that by combining VCO and EVOO, the antioxidant compounds contained in both oils may work synergistically to restrain the formation of free radicals and restore the antioxidant balance. This finding necessitates future studies to further investigate the enhanced benefit of EVOO by combining it with VCO as nutraceutical, especially to provide protection against DOX-induced cardiotoxicity.

CONCLUSION

The combination of VCO and EVOO (1:1) at 10 mL/kg of rat body weight was superior to either VCO or EVOO alone in preventing the elevation of cardiac biomarker injury in DOX-treated rats. VCO and EVOO combination was found capable of recovering cardiac histopathological alteration due to acute toxicity of DOX.

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Ethics

Ethics Committee Approval: All animal protocols were performed based on institutional guidelines for animal laboratory handling and registered under an institutional ethical clearance number of UH21020059 from the Ministry of National Education, University of Hasanuddin Faculty of Medicine, Health Research Ethics Committee.

Informed Consent: Not applicable.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Y.Y.D, Design: Y.Y.D., B.P.P., Data Collection or Processing: A.U.U., Analysis or Interpretation: Y.Y.D., A.U.U., Literature Search: A.U.U., Writing: A.U.U., Y.Y.D., B.P.P.

Conflict of Interest: No conflict of interest was declared by the authors.

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