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Eventual Medicinal Benefits of Cannabidiol Oil alleviates Doxorubicin-Related Cardiac injuries

Khadija A. Mohammed, Nabil A. Soliman, Samih I. El Dahmy and Amr A. Shalaby

Zoology Department, Faculty of Science, Zagazig University, Sharkia, Egypt
Pharmacognosy Department, Faculty of Pharmacy, Zagazig University, Sharkia, Egypt
Corresponding author: khadijahakim666@gmail.com

ABSTRACT **: Cancer and cardiovascular ailments are the two most prevalent diseases in the world. Cardiotoxicity is a feared adverse effect that could restrict how anthracycline are used in therapeutic settings. Without respect to the oncological prognosis, it may have an impact proceeding the survival and feature of being of malignance patients. A sector that remains quickly developing and holds promise intended for the medication of a wide variety of disorders is safe medical delivery using natural products. The present study's objective is assessing the efficacy of Cannabidiol (CBD) in treating Cardiotoxicity brought on by Doxorubicin (DOX). Fifty male Rats of the Sprague-Dawley breed, 150±25g each were alienated into five equal groups. Distilled water was given to Group I. whereas Group II was administered doxorubicin (18 mg/kg bwt) intraperitoneally. Group V was given Trimetazidine (10 mg/kg bwt), Group IV received 1 ml of CBD (26 mg/kg bwt), while Group III received CBD orally. On the eleventh day (IV, V), Doxorubicin (18 mg/kg bwt) was also given to both groups in a single dose. Serum was analyzed for Troponin (I), Ck-MB, ALT, AST, TNF-α, and IL-6. The pretreatment and administration of cannabidiol oil caused significant improvement in lowering cardiac enzymes (Troponin I and Ck-MB), ALT, AST and inflammatory markers (TNF-α and IL6). In conclusion, an important effect was seen after CBD (26 mg/kg bwt) treatment. Finally, the anti-inflammatory and antioxidant qualities of CBD might be the reason for its putative beneficial properties.**

KEYWORDS: Cannabidiol oil, Doxorubicin, Cardiac injuries, CK-MB, TNF-α.

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I. INTRODUCTION

Chemotherapeutic drugs are frequently utilised for healing in many malignance forms, but they can similarly have biological side effects especially non-tumour cells and frequently disrupt physiological balance in several different organs. This is primarily because oxidative stress and the generation of free radicals are frequent side effects of chemotherapy. Typically used chemotherapy prescription Doxorubicin has a history of serious adverse consequences with heart damage (Ali et al., 2017).

Doxorubicin is a documented anti-cancer antibiotic with a broad variety of activity. It is frequently used to treat cancers like leukaemia, prostate cancer, and breast cancer (Szwed et al., 2016). This medication can lead to acute and chronic cardiotoxicity, including dilated cardiomyopathy and congestive heart failure which has a dismal prognosis. Therefore, it is essential to investigate certain adjuvant medications to combat patients' dox-induced cardiotoxicity (Cardinale et al., 2020)

Doxorubicin-induced a developing issue is cardiotoxicity in childhood cancer survivors. As the overall five-year survival rates forms of infancy malignancies last to rise, the number among individuals with a cardiovascular disease risk is gradually rising. Doxorubicin has distinct effects on the developing heart than it does on the mature cardiac and now a small percentage of unprotected patients, contact during infancy results in late-onset, irreversible cardiomyopathy. Notably, late-onset toxicity is becoming more common while survival rates are getting better. Over 50,000 children cancer survivors are predicted to experience cardiotoxicity caused by doxorubicin by the year 2020, according to estimates by Mancilla et al., 2019.

Trimetazidine, a piperazine chemical, belongs to a group of drugs known as partial fatty acid oxidation inhibitors (PFox inhibitors) and is a widely used analgesic in France. In animals used as ischemia models, Trimetazidine safeguards the heart (El-Shoura et al., 2023).

Materials and Methods

1- The Scientific and Medical Research Centre (ZSMRC) of Zagazig University hosted a majority of the investigation. The updated protocol was authorised on 29 December 2021(ZU-IACUC/3/F/205/2021) by The International Animals and Use Committee's.

2- Biological Agents

Adricin (50 mg/25ml) vial, administered intraperitoneally as a single dosage (18 mg/kg bwt), was manufactured by Egypt's Badr City is home to Hikma Specialized Pharmaceuticals to be used in both (II, IV and V groups) (Moustafa and Ali 2021).

Tricardia is a film-coated tablet containing 20 mg of Trimetazidine dihydrochloride (TMZ); The TMZ was made available by Pharmaceutical Industries and Rameda Diagnostic Reagents. Tablets of Trimetazidine were crushed in a mortar with (0.06g) acacia gum was added before dilution with distilled water for group V (10 mg/kg bwt oral dosage) (Gabriel et al., 2021)

3- Substantial Preparation

Cannabidiol (CBD) was obtained by a San Diego pharmacy from California's Zova.Co, (1ml /10 mg) of Cannabidiol, and it was used as (26 mg/kg bwt) daily dosage by adding 20g of the substance to 100ml of warm distilled water and 20g of acacia gum.

4- Laboratory animals

Fifty male Sprague Dawley rats were bought from the Zagazig University's Veterinary Laboratory Animal Farm. Rats were maintained in the research laboratory site and air contact standard and housed in cages at a reduced quantity (5 rats per cage). The calculated rats received a regular diet (healthy mouse chow) and unrestricted access to water. Every animal was housed under close supervision and acclimated on behalf of 2weeks prior to starting of the experimental period under optimal conditions.

5-Animal Grouping and Experimental Design

Oral distilled water was administered to the control group. Group II was utilised for the DOX group; received take 14 days of distilled water orally before receiving just a dose of the medication (18 mg/kg bwt on the 11th day intraperitoneally, 16 hours later. Every two weeks, Group III administered (26 mg/kg bwt CBD) orally. On 11^{th} Day, a particular intraperitoneal dosage of normal saline (10 ml per kg bwt) was administered following a 16-hour wait. Group IV received both Dox and Cannabidiol oil; on day 11, they each received a single intraperitoneal injection of the chemotherapeutic medication. Group V was given Trimetazidine (10 mg/kg bwt), before giving just one intraperitoneal shot of doxorubicin, for 14 days, oral Trimetazidine (TMZ) (10 mg/kg bwt) was administered (Fig.1).

6- Lab Inspection

Blood samples were placed in non-heparinized tubing under light ether anesthesia on the last day of the dosage. Centrifugation was used to separate the serum for 20 minutes at 4000 rpm, in addition to serum was preserved at -20°C to ascertain the measures of Creatinine kinase (CK-MB),Troponin I, alanine aminotransferase (ALT), blood levels of interleukin 6 (IL-6), aspartate aminotransferase (AST), and tumour necrosis factor alpha (TNF-α). Creatinine kinas (CK-MB) was measured by spectrum kit with cat no.239000 with fixed rate method agreeing with the recommendation of the international federation of clinical chemistry (IFFC) (Tietz, 1999), Troponin I was determined using a Finecare system (cTnI rapid quantitative test) with catalog number F20315908 AD for in-vitro diagnostic use. The International Federation of Clinical Chemistry (IFCC) protocol was followed while measuring the serum activity of ALT and AST using the kinetic method and once-made packs. A fully automated analyzer, the SAT 450 system, was used, with catalogue numbers 261002 and 265002 for the ALT kit.

Analytical Statistics

Using SPSS Statistics 19, the statistical data's mean and standard deviation were used to indicate them. ANOVA was performed with the indicator levels included.

Results

1- CBD oil (26mg/kg bwt) recovers the function of cardiac enzymes.

The data in Fig.2, Table(1) demonstrate a minor improvement in heart function tests, including Troponin (I), Ck-MB, following oral CBD delivery as a preventive degree prior to receiving a single doxorubicin intraperitoneal dosage, $p<0.0001$, indicating statistical significance, when compared to the DOX group (II).

2- CBD oil acts as anti- inflammable agent

The results (Fig.3), Table (2) revealed that the Doxorubicin (G2) group had significantly higher TNF-α and IL6 levels, $P< 0.0001$ and $P< 0.0001$, respectively, for the control group. When compared to rats exposed to the toxins in the doxorubicin group and pretreatment with the medicine TMZ, respectively, the time necessary for the control group to return to normal values was significantly reduced ($P<0.0001$).

Discussion

Doxorubicin is a secondary metabolite that is a member of the anthracycline family. Dox's anti-tumor action is primarily achieved by DNA intercalation and topoisomerase II enzyme inhibition in tumours that proliferate quickly. However, Dox causes dose-dependent and cumulative cardiotoxicity (Rawat *el al*., 2021). Therefore, finding efficient therapies for DOX-induced cardiomyopathy (DIC) is essential (Kabir *et al*., 2022).

In a model of doxorubicin toxicity, the recent inquiry assesse potential beneficial therapeutic effects of cannabidiol (CBD) oil and it's anti-oxidant and anti-inflammatory function in contradiction of heart injuries. One of the proteins found in many bodily tissues is called creatine kinase, which serves as an energy source. However, the expression of creatine kinase is limited to the CK-MB isoenzyme in cardiac tissues. The ferrous iron content causes creatine isoenzyme to be damaged by Dox-induced oxidative stress, which releases the unbound radical peroxynitrite. This catalyst helps to maintain energy as ATP is the substrate used by phosphocreatine, which is made of creatine **(**Santos and Goldenberg, 2018).

Our results revealed higher levels of cardiac enzymes (Ck-MB and Troponin I) in Doxorubicin group in the vein of the findings of (Sandamali *et al*., 2020; Pan *et al*., 2022). The breakdown of myocyte cell membrane integrity is indicated by serum indicators of cardiac toxicity such as AST and ALT (El-Agamy *et al*., 2018). It is thought that increased doxorubicin metabolism-related free radical production may worsen myocyte decline and hence raise enzyme leak (Moustafa and Ali, 2021). Even though these two are not very precise biomarkers for damage to cardiac cells, the heart damage is specified by their rise in conjunction with more sensitive and specific biomarkers like cTnI (Afsar *et al*., 2017). Additionally, pretreatment with cannabidiol oil mitigates doxorubicin inducing elevation to cardiac enzymes and (ALT, AST) similar findings with Hao *et al*., 2015; Wang *et al*., 2017.

Clayton *et al*., 2021 revealed that Doxorubicin, a common chemotherapy drug, causes aortic stiffening through tumour necrosis factor alpha-mediated inflammation and extracellular matrix remodeling. Immunological responses, inflammation, bone metabolism, hematopoiesis, and embryonic development are all impacted by IL-6. The primary source of chronic inflammation is IL6, which has been connected to immunological reactions, cancer, and other disorders marked by chronic inflammation. While the cytokine storm and chronic inflammation are uncontrollable inflammatory processes, acute inflammation happens during wound healing and following an immunological response. The transcription factors signal transducer and activator of transcription 3 (STAT3) and nuclear factor-kappa B (NF-κB), as well as immune and non-immune cells and cytokines like IL-1, IL-6, and tumour necrosis factor alpha (TNF-κ), play significant roles in inflammation. The mutually beneficial relationships between NF-κB and STAT3 cause NF-κB to become hyper activated, which in turn causes the production of several inflammatory cytokines. Since STAT3 and NF-B are activated by IL-6 (Shimizu *et al*., 2021).

So our results confirmed the outcomes of Huang *et al*., (2019) and Wang *et al*., (2021), The non-psychoactive substance cannabidiol (CBD), which is extracted from Cannabis sativa, has several pharmacological actions, including the capacity to keep the heart safe and neurological arrangement, lessen pain and swelling, as well as possess anti-epileptic properties.

Final Thoughts and Future Directions

When compared to other medications (Trimetazidine), cannabidiol oil's antioxidant and anti-inflammatory qualities may be the reason for its potential heart beneficial effects. So, according to the results, the administration is essential with anthracycline chemotherapeutic agents for the prevention of irreversible damage to the heart.

Authors' assistances

The idea for the study was contributed by all authors, **N.A.S** directed the trial. **S.I.E.D, A.A.S** designed the experiments. **K.A.M** carried out this study's practical portions, analyzed data, and wrote the manuscript.

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Tables:

Table (1): Cardiac enzyme in the control and treatment groups

Treatment	CK-MB (U/L)	Troponin I (ng/mL)
Control	**15.93±0.70**	**0.001±0.00**
DOX	**70.81±1.99***	**0.026±0.001***
CBD	**17.16±2.92#**	**0.001±0.00$**
CBD+DOX	**26.71±1.28*$#**	**0.002±0.001$**

TMZ+DOX	26.41±0.84*$#	0.002±0.001*

*$# ~Mean± SD at the same column and bearing different superscripts are significantly different at P-value, P<0.0001, P<0.01, P<0.05.

Table (2): Inflammatory markers values in the control and treatment groups

Treatment	TNF-α (ng/L)	IL-6 (ng/L)	ALT (U/L)	AST (U/L)
Control	29.52±2.11	280.46±32.37	40.00±8.00	104.50±3.60
DOX	80.82±1.55*	393.94±16.51*	175.90±17.59*	299.00±13.56 *
CBD	27.85±1.37$	284.98±21.80$	31.60±8.00*$	99.40 ±4.12*$
CBD+DOX	43.18±3.51*$ #	282.44±18.78$	55.00±12.03*$#	143.20 ±24.08$#
TMZ+DOX	34.99±7.75*$ #~	255.80±15.61*$#~	173.00±7.18*#~	220.90±26.04 *$#~

*$# ~Mean± SD at the same column and bearing different superscripts are significantly different at P-value, P<0.0001, P<0.01, P<0.05.

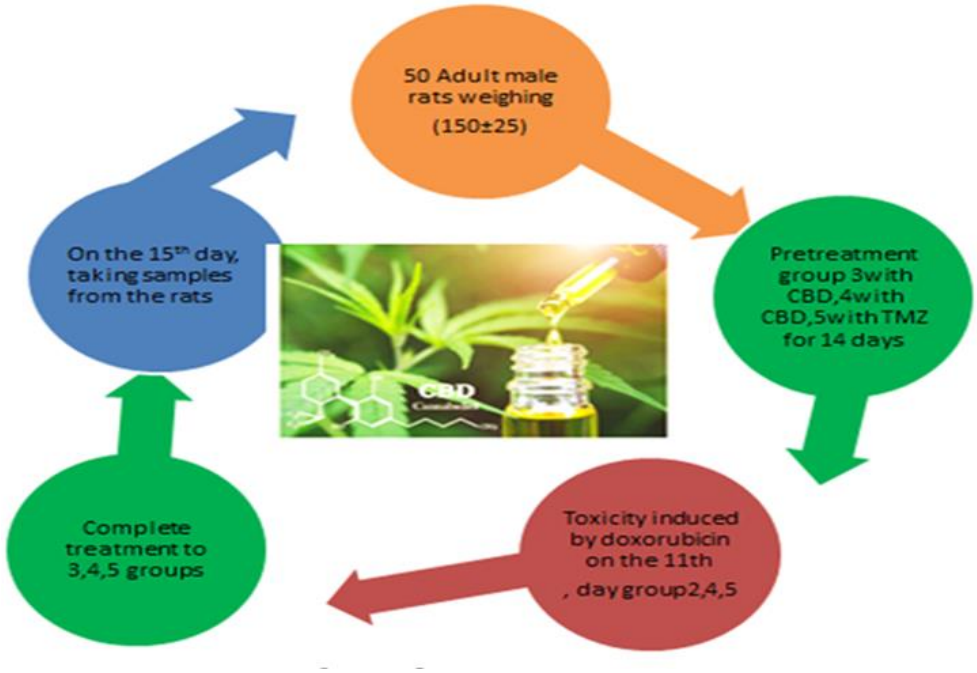
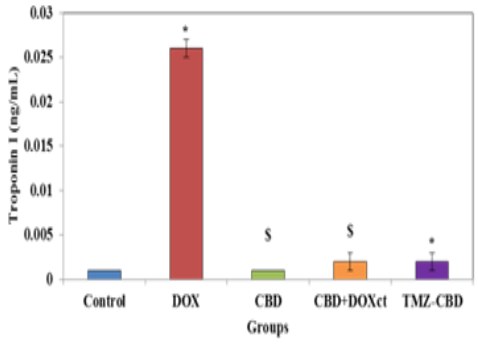


Fig.1 Experimental Design

(A)



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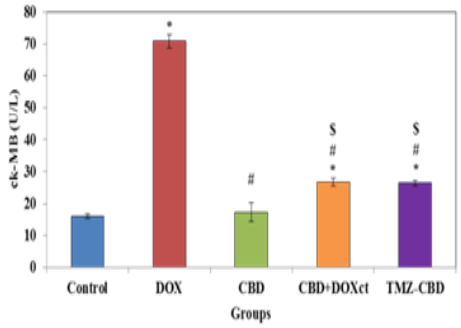

Fig. 2: The effects of TMZ and CBD combined on renal function prior to treatment. (A) Troponin I (ng/mL), (B) Ck-MB (U/L), where *, $, #, and ~ denote significant versus control, significant versus DOX, and significant versus (CBD+DOX) respectively.

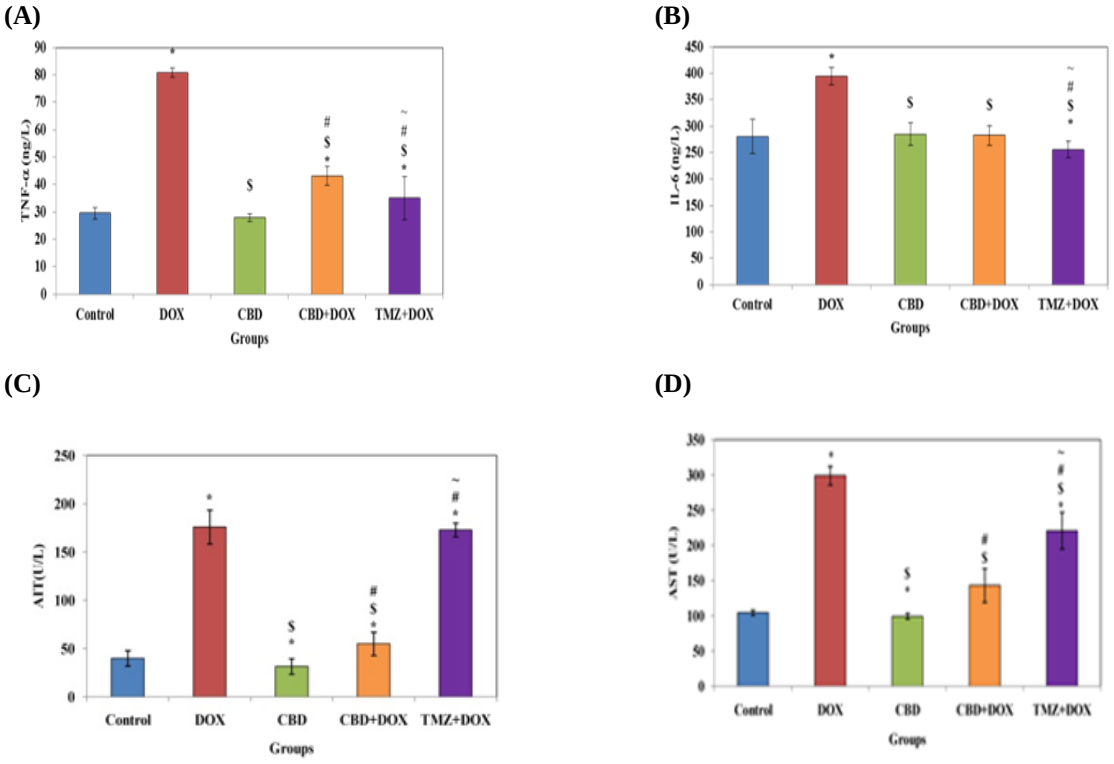

Fig. 3 Effects of CBD and drug TMZ on inflammatory markers and enzyme activities, (A) TNF-α (ng/L), (B) IL6 (ng/L), (C) ALT (U/L) and (D) AST (U/L where *, $, #, and ~ denote significant versus control, significant versus DOX, and significant versus (CBD+DOX) respectively.