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Protective Effect of Vinpocetine Against Hepatotoxicity of Doxorubicin

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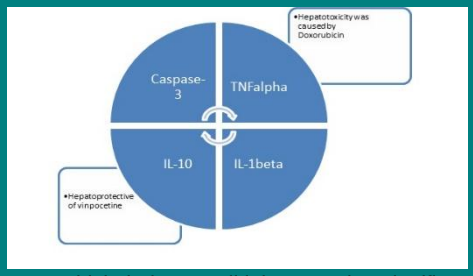
Abstract: Objective: Doxorubicin is an anti-cancer drug which induces multi-organs disorders, Doxorubicin's conventional use which result in development of cardiotoxicity, nephrotoxicity, and hepatotoxicity problems. This project was intended to explore the hepatoprotective impact of vinpocetine which is an alkaloid that has antioxidative and anti-inflammatory purposes, against doxorubicin induced liver toxicity. **Methodology:** Three groups of eighteen adult albino rats were randomly assigned: Control group had received D.W for 28 days, the second group got doxorubicin IP injection (2.5 milligrams per kilogram of body weigh t) on the 27th day, third group received vinpocetine orally at the dose (3 mg/kg /day) for 27 days. On the 27th day first give vinpocetine orally at the dose (3 mg/kg/day) and then give doxorubicin IP injection at the dose (2.5 mg/kg), after 28 days liver homogenate was prepared. **Key Findings:** The study's findings demonstrated that Doxorubicin induces cell injury causing significant rise in TNF-α levels, IL-1β, and CASP-3 and a decrease in IL-10 concentrations in liver homogenate tissues in comparison to the rats in group I as the control group. However, vinpocetine significantly ameliorated cell injury and suppressed cell apoptosis that doxorubicin induces and significantly decreases the expression of IL-1β and CASP-3 levels as well as it was raising IL-10 level when use in combination with doxorubicin in group II. **Conclusion**: The current study's findings vinpocetine inhibited doxorubicin induced apoptosis, cytokine production, which means that Vinpocetine possess role for reduction hepatotoxicity against liver damage induced by doxorubicin in rats. **Recommendation:** Study protective effect of vinpocetine and toxic effect of doxorubicin for other organs and evaluation other parameters.



Keywords: Inflammation, Hepatoprotection, Apoptosis, Liver Toxicity, Cytokines

Introduction

Cancers are a complicated large group of diseases characterized by uncontrolled abnormal cells growth. It is a multifactorial disease.[1] An innovative therapies have been developed and resulted in a considerable survival and improvement for cancer diseased patients.[2] Among the most effective anticancer drug are Anthracyclines including doxorubicin (DOX).[3]

Doxorubicin (DOX) is an antimicrobial that was found in a Streptomyces paucities mutant strain. It can bind to cell membranes and plasma proteins because it contains both hydrophobic and hydrophilic areas; moreover, additionally, DOX is amphoteric, which meaning that it performs both essential and acidic functions. These characteristics can make it a versatile chemical that can access different cellular compartments.[4]. The molecular structure of DOX consists of a tetracyclic ring with two groups that are nearby quinones, hydroquinone's, and a sugar, daunosamine that is glycosidically bound to ring A.[5] (Figure 1).

Doxorubicin has a therapeutic effect against various types of tumors like pediatric and adult solid tumors, breast cancer and sarcomas of the soft tissues and bones, ovary, thyroid and bladder. Additionally, Hodgkin lymphoma, acute myeloblastic leukemia, as well as treatment of acute lymphoblastic leukemia patients. The liposomal formulation of doxorubicin has been approved by the FDA for the treatment of multiple myeloma, patients with platinum-based chemotherapy-resistant ovarian cancer and AIDS-related Kaposi sarcoma.[6] Doxorubicin's primary mode of action involves the medication's capacity to intercalate between pairs DNA bases, resulting in DNA strand breaks and the suppression of RNA as well as DNA fabrication. Doxorubicin inhibits the enzyme topoisomerase II, resulting in apoptotic induction and DNA damage. Doxorubicin can be intracellularly reduced into a biologically-active metabolite, doxorubicinol.[7] Furthermore, it is reducible to a semi-quinone radical through many intracellular oxidoreductases. Reactive oxygen species were produced when this radical was re-reoxidized (ROS), which has been considered one of the mechanisms of DOX antineoplastic and antibiotic capabilities.[8]

Despite its therapeutic efficacy, DOX is accompanied by serious adverse effects that including hepatotoxicity, cardiotoxicity, and other organ toxicities,[9] so its clinical applications are limited. The main mechanisms of DOX toxicity through the activation of oxidative stress, reduction of topoisomerase type II, antioxidant defense mechanism attenuation, and ultimately the death of cells through necrosis or apoptosis.[10] When DOX buildup in the mitochondria, it changes the mitochondria's structure and function. However, when the cell's levels of reactive oxygen species (ROS) as well

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as reactive nitrogen species (RNS) are increased for considerable high levels, the outcome is cell damage as well as programmed cell death (PCD).[11]

It is metabolized by cytoplasmic reductases and liver microsomal enzymes, which leads to buildup of immunogenic and toxic intermediates that have been linked for production liver damage.[12]

Over metabolizing the elevation doxorubicin concentrations, there is an enormous production of ROS. As a result, ROS generate excessive damage, including damage to DNA, lipid peroxidation generation, lowering levels of vitamin E, lowering reduced glutathione (GSH) and inducing an imbalance in oxidative processes.[13] An excess of ROS (reactive oxygen species) cause nuclear factor kappaB (NFκB) is activated through the phosphorylation of IκK inhibitors as a result of IκB kinase (IκK) activation. Following that, cytokines that promote inflammation are activated by NFκB to cause apoptosis. By suppressing nuclear factor (erythroid-derived 2)-like 2 (Nrf2), a transcription factor that synchronizes cellular redox homeostasis and controls the detoxifying and antioxidant responses, DOX may promote oxidative stress.[14]

The last significant effect of doxorubicin-mediated toxicity is a reduction in inorganic phosphate concentrations, pathogenic conditions in hepatocytes which are caused by both ATP and ADP in addition to AMP. As well as liver is a major organ for metabolism in the body and play the main role in DOX metabolism so that it may be more affected by it toxicity and about Forty percent of patients receiving the medication had some kind of liver damage.[13] Hence, it is necessary to investigate an active agent against liver damage induced by Dox. So with the development of modern pharmacology and molecular biology and due to the relation between Pharmacology and pharmacognosy and some natural products that have important pharmacological effects for the prevention and treatment of human diseases among these alkaloids were considered as the most commonly encountered.[15] Among the several natural substances with substantial pharmacological significance, the most common substances are alkaloids. For example, the Apocynaceae family of plants contains more than 2500 distinct forms of alkaloids.[15] Vinblastine and vincristine are belong to vinca alkaloids and are well-known antineoplastic agent and the plants in this family with the most striking visual appeal. Vinca minor (genus Vinca) is Another member of this family of plants that has vincamine, which also contains numerous other vinca alkaloids, such as vinpocetine.[16]

The alkaloid vinpocetine, was discovered in the Vinca minor periwinkle plant leaves.[17] Vinpocetine (Cavinton®), which was initially sold in Hungary in 1978, has been used extensively throughout Asia and Europe to treat memory problems, strokes, and senile dementia. Vinpocetine-containing products which are currently utilized as dietary supplements all over the world.[18] Apovincamine acid (AVA) is vinpocetine's major de-esterified active metabolite (Figure 2).[19, 20]

Until today, there are no reports indicating that vinpocetine usage at a therapeutic level has been connected to any side effects, toxicities, or contraindications. Make it unique chemical to investigate its potential for therapeutic use.[21] The pharmacological effects of vinpocetine are numerous.[22]

There are several important modes of action for vinpocetine, the main one is the anti-inflammatory effect by IκK inhibition, which in turn inhibits nuclear factor-(NF-κB) activation and translocation, the primary signaling pathway for the production of inflammatory cytokines and gene expression is NF-κB.[23]

Furthermore, it exerts antioxidant effects via reducing ROS, eliminating free radicals, (altering the metabolism of dopamine, and reducing vascular thrombosis).[24, 25] Moreover, Vinpocetine provides dose-dependent prevention against liver damage which causes by cisplatin.[26]

Based on these findings, we hypothesized that Vinpocetine may provide some protection against Dox-induced hepatotoxicity. And the purpose of this research was for verification of this theory.

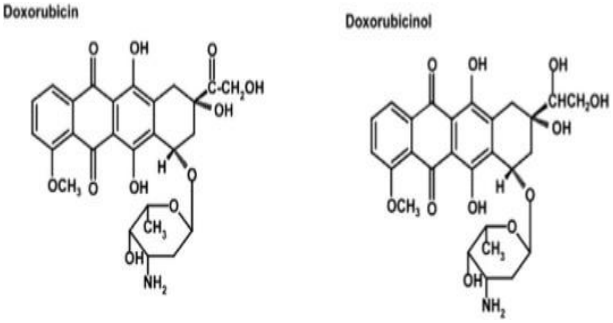

Figure (1): The chemical composition of doxorubicin with its metabolite doxorubicinol.[27]

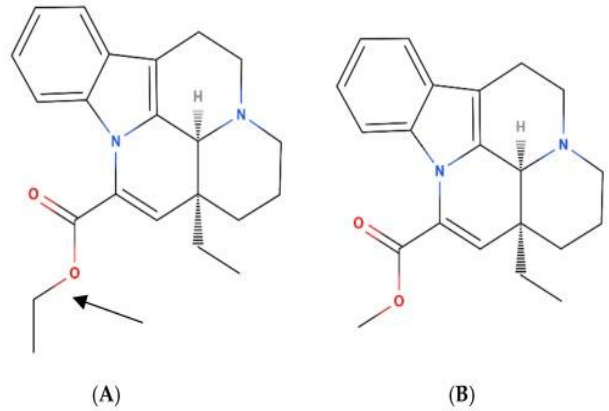

Figure (2): Vinpocetine's and the vinca alkaloids' chemical structures (A) and apovincamine (B).[28]

Materials and Methods

Materials and Chemicals

All chemicals and drugs utilized in this study were of a good available source. And all kits that used were of high specificity and sensitivity that are listed down in a table (1) with their producers and their suppliers.

Table (1): Materials and Chemicals with their suppliers.

Materials and Chemicals	Suppliers
Doxorubicin (DOX) [50mg / 25ml] vial	EBEWE Pharma, Austria
Vinpocetine [capsule, 10mg]	America Medic & Science (AMS) (USA)
Normal saline [0.9%] solution	Pioneer, Iraq
Distilled water (D.W.)	Pioneer, Iraq
Sulfuric acid	Pioneer, Iraq
Diethyl ether	Pioneer, Iraq
Phosphate buffered saline solution	Pioneer, Iraq
CASP-3 ELISA Kit for Rat.	Elabscience, USA
ELISA Kit for Rat Interleukin 1 beta (IL-1β)	Elabscience, USA
Interleukin 10 (IL- 10) ELISA Kit for Rats	Elabscience, USA
ELISA Kit for Rat Tumor necrosis factor - alpha (TNF-α)	Elabscience, USA

Methods

Preparation of Doxorubicin Solution: Doxorubicin (DOX) vial (50mg / 25ml) was diluted with 0.9% normal saline (NS) to be injected intraperitoneally (IP) at a dose 2.5 mg/kg; where (1.25ml of DOX vial was reconstituted with 1ml of normal saline, dose of each animal 0.2 ml of diluted DOX).[29]

Animals: Eighteen three-month-old, healthy, male and female albino rats weighing between 180 and 250 grams. These animals maintained in the Animal House under were utilized in this study circumstances with regulated temperature 25°C. Throughout the course of experiment, the animals were drink tap water and the fed commercial pellets**.**

Experimental Design: The three groups (each with six animals) of healthy rats were randomly assigned:

GROUP I: rats received D.W alone orally for 28 days (control group).

GROUP II: rats IP injected with doxorubicin (DOX) in single dose (2.5 mg/kg/1.25 which diluted with 1ml of normal saline) on the 27th day.[29].

GROUP III: rats in this group received vinpocetine orally at the dose (3 mg/kg/day) for 27 days. On the 27th day first give vinpocetine orally at the dose (3 mg/kg/day) and then give doxorubicin IP injection at the dose (2.5 mg/kg/1.25 which diluted with 1ml of normal saline).[30-32].

At the end of treatment (at 28 day) diethyl ether was used to euthanized each animal**.**

Preparation of liver Tissue Homogenate Sample

In order to prepare the liver tissue homogenate, extra blood had to be rinsed off using ice-cold phosphate buffer saline (PBS), with a pH of 7.4, subsequently, the liver tissue was dried using filter paper, and its weight was recorded before homogenization. after which, the liver tissue from each rat was finely chopped and placed in a 15 ml plastic test tube with a cooled PBS solution pH is 7.4; (weight of tissue (gram): volume of PBS is 1.9 ml).

conduct homogenization. Subsequently, a 20-minute centrifugation at 3000 ×g was performed on the homogenate. The supernatant had been meticulously gathered and kept at 20°C till the estimation time to the different cytokines: IL- 1beta, TNF-α, IL- 10 and CASP-3 levels.

Principle for Estimating the TNF-α levels: The principle of the test depends on the utilization of Sandwich-ELISA as the methodology. An antibody specific to rat TNF-α is already pre-coated on the plate of micro-ELISA kit. Each of the standard or sample is combined with the particular Ab and inserted onto the appropriate plate wells of micro-ELISA. Subsequently, an antibody for biotinylating detection tailored for rat TNF-α with a combination of Avidin and Horseradish Peroxidase (HRP) are added to each microplate well. The plates are then incubated.

Free components are removed after incubation. Then, the substrate reagent is introduced into every well, just the wells containing rat TNF-α, the conjugate of biotinylated detection and Avidin-HRP will show up as blue.

To halt the enzyme substrate reaction, stop solution will be added, and the reaction will appear yellow. Quantifying the optical density (OD) can be done at a wavelength of 450 nm ±2 nm using spectrophotometry.

Rat TNF-α concentration is correlated with the optical density (OD) value. The concentration of rat TNF-α in a sample can be ascertained by comparing its optical density (OD) with the standard curve. The level of IL-1β in each liver tissue homogenate was expressed as Pg/mL.[33]

Estimation of Interleukin-1 Beta (IL-1β) level Principle

With this kit for ELISA, the technique of ELISA sandwich is employed. Pre-coated plate of micro-ELISA with a rat IL-1β specific antibody utilized in this kit. Each of the standard or sample is put to the proper wells of micro-ELISA plates and combined with the appropriate antibody. Then, a biotinylated antibody which is specific for the detection of rat IL-1β and the conjugate of HRP (Avidin Horseradish Peroxidase) are added to each microplate well. The plates are then incubated. Following incubation, free elements are eliminated through washing. Then, each well is filled with the substrate reagent; the only wells holding the rat IL-1β, the conjugate of Avidin-HRP and biotinylated detection antibody will both turn blue. By adding a stop solution (sulphuric acid), the reaction of enzyme and substrate will be stopped and will turn yellow.

Quantifying the optical density (OD) can be done at (450 ± 2) nm wavelength using spectrophotometry. The value of OD and the rat's IL-1β concentration are directly connected. One can determine the amount of rat IL-1βpresent in a sample by comparing its optical density to the standard (OD) curve. The IL-1β level in each liver tissue homogenate was expressed as Pg/mL.[34]

Estimation of Interleukin-10 (IL-10) level Principle

The technique applied with this ELISA kit was Sandwich-ELISA. The plate of the kit is already covered in an antibody that is specific to rat IL-10. The specific antibody is then added once the matching plate wells of micro-ELISA have been filled with either the standard or sample.

Next, an antibody for biotinylating detection tailored for rat IL-10 with a combination of Avidin and Horseradish Peroxidase (HRP) are added to each microplate well, and it is then incubated. Free components are removed after incubation.

After that, each well receives an addition of substrate reagent; only the rat IL-10-containing wells, blue coloration will be produced by the biotinylated detecting antibody and the Avidin-HRP conjugate.

By adding stop solution (sulfuric acid), the substrate reaction of an enzyme will be stopped and will turn yellow. 450 nm ± 2 nm is the wavelength at which a spectrophotometer can measure the optical density (OD).

The OD value and the concentration of rat IL-10 are directly correlated. Through a comparison of the standard curve with the sample's OD, one can determine the rat's IL-10 value that present in the sample. Pg/mL was used to express the amount of IL-10 in each liver tissue homogenate.[35]

Estimation of CASP-3 level Principle

The principle of the test depends on the utilization of ELISA Sandwich method. An antibody (Ab) specific to rat CASP3 has already been pre-coated on the plate of micro-ELISA included with this kit.

A combination of standard or sample and the appropriate antibody is put to the proper wells for plates of micro-ELISA. After that, an antibody for biotinylation detection particular to the rat conjugate is inserted into each well of microplate, and it is incubated.

After incubation, free parts are removed with a wash. Following that, each well receives an addition of substrate reagent; only wells containing rat CASP3, blue coloration will be produced by the biotinylated detecting antibody and the Avidin-HRP conjugate.

The substrate reaction of an enzyme is stopped by a stop solution addition, such as sulfuric acid, which appeared to be yellow. 450 nm ± 2 nm is the wavelength at which a spectrophotometer can measure the optical density (OD).

Rat CASP3 concentration is correlated with the OD value. Through a comparison of the standard curve with the sample's OD, one can determine the concentration of rat CASP3 present in the sample. The level of CASP3 in each liver tissue homogenate was expressed as ng/mL.[36]

Statistical Analysis

The mean's standard error, and mean values were used to express the data. To determine if the three groups differed significantly from one another, the unpaired Student t-test was employed for comparing between two groups. ANOVA, or one-way test for variance, was used to assess the statistical significance for the groups differences. Statistical significance was indicated by P value less than 0.05 for differences.

Results and Discussion

Doxorubicin's consequence on the TNF-α (tumor necrosis factor-alpha) level in tissue homogenate for rat liver. As shown in Tables (2 and 3), and Figure (3) Doxorubicin significantly increased (P less than 0.05) the level of TNF-α in liver homogenate of rats of Group II (given 2.5 mg/kg doxorubicin (IP)) in comparison to Group I (control group). The means and standard error values (mean ± SE) of TNF-α level in (Group I) and (Group II), were, 255 ± 27.17 and 748.33 ± 35.44, subsequently.

Additionally, there were significantly higher TNF-α levels were observed in the liver homogenate in Group III that received intraperitoneal doxorubicin at dose of 2.5 mg/kg and vinpocetine orally at the dose (3 mg/kg/day)) in comparison to the level in (Group I) as shown in Table (4). This result revealed that vinpocetine did not significantly reduce the expression of TNF-α induced by doxorubicin Table (5). The concentration of TNF-α in (Group III) and (Group II) were, mean ± SE, 769.16 ± 60.03 and 748.33 ± 35.44 subsequently.

The impact of doxorubicin on the level of interleukin-1beta in the homogenate of rat's liver tissue

Table (2 and 3) and Figure (4) demonstrated that in Group II (rats had been received Doxorubicin) their liver tissue homogenates showed a considerable arise in the IL-1β levels (P value less than 0.05) in comparison with Group I (control group). The (mean ± SE) of this particular cytokine in liver homogenate for (Group II) and (Group I) were subsequently, 390 ± 47.36 and 191.66 ± 10.46.

However, Table (5) revealed that the presence of vinpocetine in (Group III) (Rats given doxorubicin (DOX) intraperitoneally at dose of (2.5 mg/kg) and vinpocetine orally at the dose (3 mg/kg/day)) attenuated the inflammatory effect of doxorubicin and a statistically considerable decreasing (P value less than 0.05) with the expression of IL-1β in this group's liver homogenate than the conformable levels in the liver homogenate tissue of (Group II) that received Doxorubicin (2.5 mg/kg) intraperitoneal injections. The (mean ± SE) of (IL-1β) levels in (Group III) and (Group II) were 221.66 ± 13.64 and 390 ± 47.36 respectively.

Doxorubicin effect on interleukin - 10 in tissue homogenate for rat liver. Data presented in table (2 and 3) Figure (5) revealed the pro inflammatory effect of DOX that there was a considerable (P<0.05) decrease in the tissue homogenate concentrations of IL-10 for liver in the Group II of rats which received of Doxorubicin (2.5 mg/kg) injected intraperitoneally (IP) in the 27th day in comparison to control Distilled water (Group I) rats. The mean and standard error of the mean (SE) (IL-10) levels in liver tissue homogenate were, subsequently 703.33 ± 44.95 and 1216.66 ± 94.28.

While as seen in Table (5) the addition of vinpocetine in (Group III) (treated with vinpocetine + doxorubicin) ameliorated the inflammation and there was a considerable rise in IL-10 levels (P value less than 0.05) in the homogenate of liver's tissue in comparison with (Group II). The mean and standard error value of IL-10 levels in homogenate tissue of liver in (Group III) and (Group II) were 946.66 ± 80.43 and 703 ± 44.95 respectively.

Doxorubicin effect on CASP-3 in homogenate for rat liver. Table (2 and 3) and Figure (6) demonstrated Apoptosis Induced by Doxorubicin and in comparison, to control (Group I) rats, rats were given Doxorubicin (2.5 mg/kg) (Group II) revealed a statistically significant rise (P value less than 0.05) in the levels of CASP-3 in liver homogenate tissue. The respective means and standard error values of such cytokines in liver tissue homogenate of (Group I) and (Group II) were subsequently, 2.23 ± 0.23 and 7.01 ± 0.64.

Additionally, Table (5) and Figure (6) demonstrated the attenuation effect of vinpocetine on apoptosis induced by DOX when there was a considerable (P<0.05) reduction in the CASP-3 levels of homogenized tissue for the Group III (rats treated doxorubicin (DOX) intraperitoneally at dose of (2.5 mg/kg) and vinpocetine orally at the dose (3 mg/kg/day)) in comparison to corresponding levels in the group of rats given Doxorubicin (2.5 mg/kg) IP injections (Group II). In liver tissue homogenate, mean ± SE of CASP-3 levels in (Group III) and (Group II) were, 4.25 ± 0.58 and 7.01 ± 0.64 subsequently.

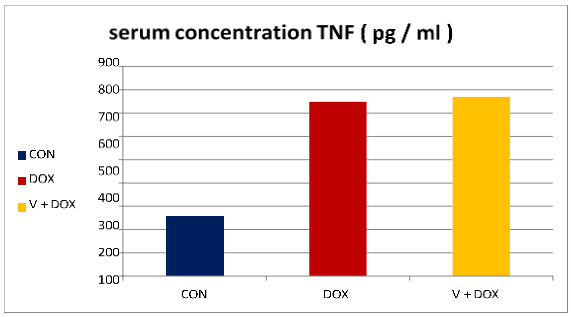

Figure (3): Effect of doxorubicin and combination of doxorubicin and vinpocetine on level of TNF in liver tissue homogenate of rat.

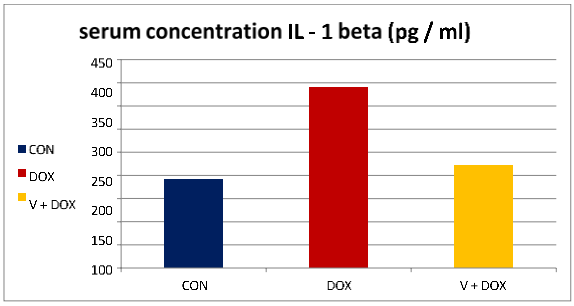

Figure (4): Effect of doxorubicin and combination of doxorubicin and vinpocetine on level of IL-1β in liver tissue homogenate of rat.

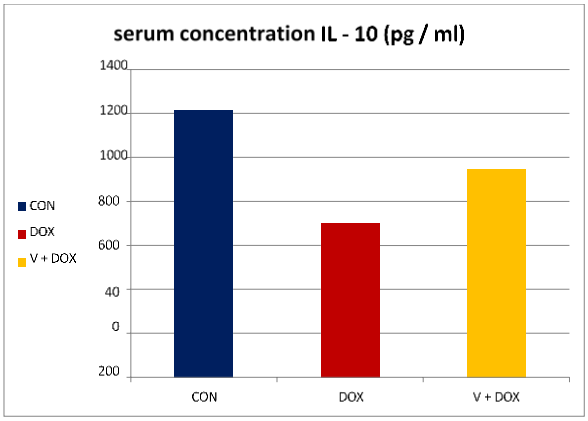

Figure (5): Effect of doxorubicin and combination of doxorubicin and vinpocetine on IL-10 level in rat liver tissue homogenate.

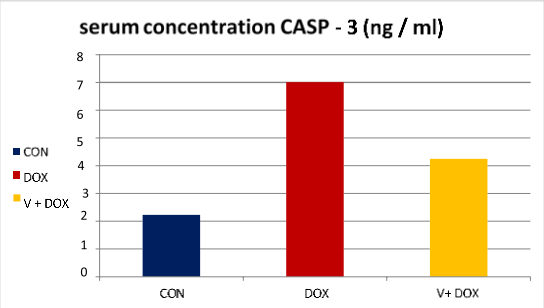

Figure (6): Effect of doxorubicin and combination of doxorubicin and vinpocetine on CASP-3 level in rat liver tissue homogenate.

Table (2): Mean and Standard Error of (IL-10), (IL-1β), (TNF-α) and CASP - 3 levels in liver tissue homogenate of group I, II and III.

groups	treatment	IL-10 [pg/ml] ± SE	IL-1β [pg/ml] ± SE	TNF-α [pg/ml] ± SE	CASP-3 [ng/ml] ± SE
I	Control / D. W	1216.66 ± 94.28	191.66 ± 10.46	255 ± 27.17	2.23 ± 0.23
II	Doxorubicin	703.33 ± 44.95	390 ± 47.36	748.33 ± 35.44	7.01 ± 0.64
III	Vinpocetine + doxorubicin	946.66 ± 80.43	221.66 ± 13.64	769.16 ± 60.03	4.25 ± 0.58

Table (3): Comparison in Mean and standard Error of (IL-10), (IL-1β), (TNF-α) and CASP-3 levels between group I and group II.

Groups		N	Mean	SE	P Value
IL1B	Group I a) Group II b)	6 6	191.67 390	10.56 47.36	<0.001
IL10	Group I Group II	6 6	1216.67 703.33	94.28 44.96	< 0.001
CASP	Group I Group II	6 6	2.23 7.02	0.24 0.64	< 0.001
TNF	Group I Group II	6 6	255 748.33	27.17 35.44	< 0.001

a) Group I: rats orally administration D.W alone; b) GROUP II: rats received doxorubicin.

Table (4): Comparison in Mean and standard Error of (IL-10), (IL-1β), (TNF-α) and CASP-3 levels between group I and group III.

Groups		N	Mean	SE	P Value
IL1B	Group I a) Group III C)	6 6	191.67 221.67	10.46 13.64	0.477
IL10	Group I Group III	6 6	1216.67 946.67	94.28 80.43	0.024
CASP	Group I Group III	6 6	2.23 4.25	0.24 0.58	0.015
TNF	Group I Group III	6 6	255 769.17	27.17 60.03	< 0.001

a) Group I: rats orally administration D.W alone; C) GROUP III: rats received vinpocetine and doxorubicin

Table (5): Anti- inflammatory and antiapoptotic effects of vinpocetine.

Groups		N	Mean	SE	P Value
IL1B	Group II b) Group III C)	6 6	390 221.67	47.36 13.64	< 0.001
IL10	Group II Group III	6 6	703.33 946.67	44.96 80.43	0.039
CASP	Group II Group III	6 6	7.02 4.25	0.64 0.58	0.002
TNF	Group II Group III	6 6	748.33 769.17	35.44 60.03	0.738

b) GROUP II: rats received doxorubicin; C) GROUP III: rats received vinpocetine and doxorubicin

Discussion

One common chemotherapeutic drug which used to treat a number of malignancies is doxorubicin, include malignancies of the lung, thyroid, ovary, breast, lymphomas, and leukemia. It is a member of the anthracyclines class of chemicals.[37]

Doxorubicin's harmful effects on multiple organs, including the formation of type-2 diabetes, hepatotoxicity, nephrotoxicity, cardiotoxicity, and diabetic cardiomyopathy, so its conventional use has been restricted.[38]

Both subacute and acute dosages of doxorubicin have been shown to produce liver damage because of its biphasic nature.[39] The primary molecular mechanism by which doxorubicin causes hepatotoxicity is generation of ROS while it is being metabolized in the liver.

This leads to an unbalanced redox potential, which in turn causes oxidative stress, decreased antioxidant enzyme levels, mitochondrial dysfunction, inflammation, and apoptosis. The primary organ engaged in detoxification and metabolism of pharmaceuticals is the liver.[40]

In this study we demonstrated that doxorubicin may induce oxidative stress by increase the key mediators of pro-inflammatory signaling pathway triggered by cytokines through causing an elevation in the TNF-α and IL-1β in doxorubicin treated group with considerable deviation (p value less than 0.05) in comparison with Group I (control group) table 3). This is in agreement with other studies that predicted that doxorubicin have the ability to increase TNF-α and IL-1β levels, showing that doxorubicin had an impact in triggering an inflammatory activity in rat liver tissue.[41]

On the other hand, A statistically significant (p value less than 0.05) deviation was seen in the anti-inflammatory cytokine interleukin-10 level between the experimental group (Group II) and the control group as shown in table (3). Doxorubicin might be induced this reduction and that is parallel to another study that predicts that doxorubicin will reduce IL-10 level.[41]

As that the important mark of apoptosis is the production of CASP-3.[42] According to this recent study, doxorubicin causes an elevation in the CASP-3 expression in the rat liver tissue in which ($p< 0.05$) and the result was statistically significant in comparison to control group table (3). This was consistent with another studies reported that the doxorubicin induces cell apoptosis and thus increases CASP-3.[43]

Ethyl apo-periwinkle-22-acetate, another name for vinpocetine, is a highly lipophilic vincamine indole alkaloid that easily crosses the blood-brain barrier to reach brain tissue. [44] Its antioxidant qualities have been proven in vitro as well as in vivo.[31, 45-47] Also it has anti-inflammatory properties, neuroprotection and delays the aging process. And the use of vinpocetine in this study revealed these antioxidant and anti-inflammatory effects when vinpocetine significantly decreased ($p<0.05$) the concentrations of expression to reduce pro-inflammatory cytokines (interleukin-1beta (IL-1β) and significantly increase (IL-10) level (p value less than 0.05) in comparison to doxorubicin group table (5). These results were paralleled with another study predicting that vinpocetine has antioxidant effect and produce increase anti-inflammatory cytokine and reduced the pro-inflammatory cytokine.[48]

On the other hand, this study showed that vinpocetine in Group III (doxorubicin vinpocetine) did not reduce the level of TNF-α in contrast to the doxorubicin-treated group table (5), and the amount was noticeably higher than that of group I (control group) as shown in table (5).

This result was inconsistent with another studies which predicted that vinpocetine has no effect on the TNF-α.[48] The reason for this may be the short period of treatment or the low dose, therefore, the dose can be increase above 3 mg/kg according to other research that use 10 mg/kg.[49]

The recent studies revealed Vin's hepatoprotective properties included anti-inflammatory, anti-oxidative, and anti-apoptotic properties.[26] and as the level of CASP-3 is an important mark of apoptosis.[42]

In this work vinpocetine showed this antiapoptotic effect by producing a considerable reduction (p value less than 0.05) in

the rat liver tissue's CASP-3 expression in comparison with doxorubicin treated group table (5). This resembling the result of another studies predicting that vinpocetine decreased the CASP-3 expression.[48]

This study clarifies the effect of doxorubicin and vinpocetine on the IL-1β, TNF-α, IL-10 as well as CASP-3 concentrations.

Conclusions

It could be concluded that vinpocetine has role for reduction liver damage effect against doxorubicin induced hepatotoxicity of rat, via its inhibitory effect on cytokine production and apoptosis, therefore, for preventing doxorubicin -induced hepatotoxicity, vinpocetine looks to be a potential treatment.

ABBREVIATIONS

CASP-3 Caspase- 3

DOX Doxorubicin

OD Optical density

IL-10 Interleukin-10

IL-1β Interleukin-1 Beta

TNF-α Tumor Necrosis Factor-Alpha

SE Standard Deviation

Disclosure Statements

- **Ethics approval and consent to participate:** This study was approved by the Scientific and the Ethical Committees of the College of Pharmacy / Basra University. according to document number EC54 on April 15, 2024, to get this approval.
- **Availability of data and materials:** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. Any materials used in the study can also be provided upon reasonable request to the corresponding author.
- **Consent for publication:** Not applicable. This study did not involve human participants, and no personal or identifiable human data are included in this manuscript.
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