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Carboplatin and thalidomide induced neuro-hepatotoxicity in rats: Diminish effect of *Citrullus colocynthis*

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ABSTRACT
Carboplatin and thalidomide are good candidate's treatments against many types of cancer. Latest studies demonstrated that these chemotherapeutic drug cause oxidative damage. So, the present study was conducted to estimate the neurotoxic and hepatotoxic effects induced by carboplatin/thalidomide regimen and to explore the potential protective effect of *Citrillus colocynthis* extract (CCE) in rats. The obtained results showed that carboplatin/thalidomide induced histological changes, caused significant elevation in serum liver enzymes, decline in brain and plasma neurotransmitters, and increase in acetylcholine esterase. At the molecular level, carboplatin/thalidomide caused marked elevation in inflammatory and apoptotic markers and suppressed the expression of proliferator-activated receptor-gamma coactivator-1 and mitochondrial transcription factor a in brain and liver tissues. Also, the regemin declined the GSH system and nuclear factor erythroid-2-related factor 2 (NRF2), and induced oxidative stress as indicated by elevated tissues level of thiobarbituric acid reactive substances and 8-hydroxydeoxyguanosine. The co-supplemnation with CCE minimized thalidomide/carboplatin-induced abnormalities in brain and liver tissues. As a result, the current findings revealed that CCE may have a protective effect against carboplatin/thalidomide-induced toxicity.

Keywords: Carboplatin; Thalidomide; *Citrullus colocynthis*; Neurotransmitters; Brain and liver; male rats.

1. Introduction

Chemotherapy can be used for therapeutic purposes (almost involving a combination of drugs), or it can aim at prolonging life or reducing symptoms (palliative chemotherapy) (Devita *et al.*, 2001). Chemotherapeutic agents cause frequent and predictable multisystem toxicity. The acute toxicities are induced immediately post-therapy and are usually reversible while the long-term toxicities are delayed and irreversible (Remesh, 2012).

Platinum-based combination chemotherapy is first-line therapy in many types of cancer especially lung cancer (Flotten et al., 2012). It is speculated that carboplatin anti-tumor activity is the result of the formation of

platinum (Pt) DNA adducts. Since myelosuppression is a limitation of carboplatin clinical application, therefore, great efforts are done to minimize carboplatin side effects and allow its high dose adminstration. Many drugs are paired with carboplatin during the treatment of cancer including microtubule-targeted agents (paclitaxel, docetaxel, or vinorelbine), DNA damaging agents (gemcitabine or irinotecan), and immunomodulatory agents (thalidomide) (Korst *et al.,* 1997).

Antiinflammatory and immunomodulatory compounds such as thalidomides and its analogs can be used to treat autoimmune diseases, multiple myeloma, prostate cancer, pancreatic cancer and lung cancer (Sherbet, 2015). The boundless uses of carboplatin/thalidomide mix raised worries about their expected hepatotoxicity and neurotoxicty. Chemo mind; post-chemotherapy intellectual weakness, is a neurological condition described by an abatement in more elevated level psychological and chief capacity after the organization of malignancy treatment system (Raffa *et al*., 2006). The hidden reasons for chemo cerebrum are not surely known yet, components that have been proposed incorporate chemotherapy-actuated DNA harm, disturbance of vascular blood stream in the mind, incendiary reactions to receptive oxygen species, and impedance of synapse signals (Scheibel *et al*., 2004).

The reports on the carboplatin/thalidomide combination potential toxicities have grown along with their usage. Since chemotherapeutic agents are accumulated and metabolized primarily in the liver after they enter the bloodstream, therefore understanding their adverse effects on hepatocytes is essential (Remesh, 2012). Data on the molecular targets of carboplatin/thalidomide combination is still largely inadequate. A better understanding of this subject is highly important to suggest the best methods of protection against the hepatotoxic effects of carboplatin/thalidomide combination.

. The usage of antioxidants through chemotherapy may improve treatment by lowering the production of oxidative stress (Conklin, 2004). Cell reinforcements are amazing foragers of free extremists and fill in as inhibitors of neoplastic cycles. Many artificial and natural antioxidants have beneficial effects on human health and disease inhibition (Bagchi *et al*., 2010). *Citrullus colocynthi*s extract is utilized generally in assorted pieces of the world for the therapy of various ailments, including diabetes, asthma, bronchitis, jaundice, joint torment, malignant growth and mastitis (Asyaz *et al*., 2010). Citrullus colocynthis fruit contains a variety of bioactive chemicals. They are arranged as glycosides, flavonoids, alkaloids, carbs, unsaturated fats and fundamental oils (Salama, 2012). The current study aimed to evaluate the carboplatin/thalidomide -induced hepatotpxicty and neurotoxicity and to assess the possible protective effect of *Citriullus colocynthis* extract (CCE) against these toxicities.

2. Material and methods

2.1. Tested compounds and doses

The doses of thalidomide (from Sigma Chemical Company), carboplatin (from Vitafor NV/SA) and *Citrillus colocynthis* extract (CCE) were given according to the studies of Srikanth *et al*. (2008), de Souza *et al*. (2014) and Ayyad *et al*. (2012), respectively.

2.2. Preparation of Citrillus colocynthis

The fruit of *Citrillus colocynthis* was used in which 100 gm wet fruit was dissolved in 100 ml distilled water, boiled for 5 minutes, homogenized, filtered then lyophilized. The used extract was prepared by dissolving 10 gm of the lyopholyzed water extract in 1 L water (Hamad *et al*., 2015).

2.3. Animals and experimental design

Twenty male Wistar rats weighing 185-200 g (12 weeks old) were used in the present study. Animals were received from Alexandria University's Faculty of Medicine. The experiment as approved by the local ethical guidelines of Institutional Animal Care & Use Committee (IACUC), Alexandria University, Egypt (AU14-21026-2-4) and all the methods were performed according to the guidelines and regulations of the same Committee. The animals were fed a basic meal and had unlimited access to tap water. Animals were separated into four equal groups (5 animals per each group) after two weeks of acclimatisation. The first group 1 was utilized as control, the second was dealt orally for 28 sequential days with *Citrillus colocynthis* extract (100 mg/kg BW), the third was dealt orally every day with thalidomide (30 mg/kg BW) and carboplatin intraperitoneally (i.p.) week by week (60 mg/kg BW) and the fourth was treated with thalidomide, carboplatin and CCE.

2.4. Blood and tissues collections and preparation

Toward the finish of the 28th day of the trial time frame, all animals were forfeited utilizing isoflurane inhalation and blood tests were gathered in test tubes containing heparin as an anticoagulant. To obtain plasma, the blood samples were centrifuged at 860 ×g for 20 min for the partition of plasma which was kept at – 80 °C until

investigations of the tried boundaries. Cerebrum cortex and liver tissues were quickly taken apart out, washed utilizing chilled saline arrangement (0.9%), and eliminated the following fat and connective tissues. The tissues were divided into 4 aliquots; the first one was used for DNA isolation for the assessment of 8-hyroxy-deoxy-guanosine (8-OH-dG), the second for total RNA isolation for the assessment of gene expression, and the third aliquot was homogenized in ice-cold sucrose buffer (0.25 M, pH 7.4) in ratio1:10 then homogenates were centrifuged at 10000×g for 10 min at 4 C̊. The obtained supernatants were stored in aliquots for subsequent determinations. The last aliquot was used for histopathological analyses.

2.5. Biochemical parameters

Biosystems S.A. Costa Brava 30, Barcelona, Spain commercial kits were used to estimate various biochemical parameters (AST, ALT, ACP, ALP, Albumin and Total protein) and neurotransmittes (serotonin, dopamine and norepinephrine levels and acetylcholine esterase).

2.6. Tissue content of thiobarbituric acid-reactive substances

Lipid peroxidation was assayed using thiobarbituric acid-reactive substances assay (Draper and Hadley, 1990).

2.7. Tissue content of 8-OH-2-deoxygunaine (8-OHdG) as index of oxidative DNA damage

The determination of 8-OHdG in the tissues DNA starts with the isolation of genomic DNA using DNeasy kit (Qiagen Inc, USA.) then Nanodrop was used to determine its concentration. 8-OH-dG ELISA kit (cat. no. ab201734; Abcam) was used for experimentation of the resultant DNA.

2.8. Tissue content of reduced glutathione

Reduced glutathione (GSH) content was estimated according to Griffith (1980). The rate of formation of TNB was monitored by recording the rate of absorbance change at 412 nm.

2.9. Tissue content of nuclear factor-Kappa B and nuclear respiratory factor 2

The contents of nuclear factor-kappa B and nuclear respiratory factor 2 (NRF2) in tissues homogenates were estimated using rat ELISA kit (cat. No: CSB E13148R, and CSB EQ027869RA, respectively) (CUSABIO, China).

2.10. Assay of caspase-3 activity

The caspase-3 enzymatic activity was assayed using Caspase-3 Assay Kit (Elabscainces, USA).

2.11. Gene expression analysis using RT-PCR

Quantitative analyses of gene expression of peroxisome proliferator activator receptor gamma-coactivator 1α (PGC-1α) (Li *et al*., 2011) and mitochondrial transcription factor A (mtTFA) (Piantadosi and Suliman, 2006) in brain and live tissues were performed using quantitative real time reverse transcriptase-polymerase chain reaction (qRT-PCR). The total RNA was extracted from the studied tissue using RNeasy Mini Kit (Qiagen, Germany). The reverse transcription of the extracted RNA was done using QuantiTect Reverse Transcription Kit (Qiagen, Germany). The obtained cDNA then amplified and detected using specific primers for PGC-1α, mtTFA, and GAPDH (Table 1) by qRT-PCR assay using Rotor-Gene SYBR Green PCR Kit (Qiagen, Germany). Rotor-Gene Q-Pure Detection version 2.1.0 (build 9) (Qiagen, Valencia, CA, USA) was used to determine the Threshold cycle (Ct), which is the point where the instrument first detects fluorescence above background noise. For each gene, the expression was calculated relative to GAPDH as a reference gene using the formula: $2^{-\Delta\Delta Ct}$ (Livak andSchmittgen, 2001).

Table 1: Primer sequences for RT-PCR

Gene	Accession number	primer sequence	
PGC1-α	NM_031347.1	F:	5'- GTGCAGCCAAGACTCTGTATGG -3'
		R:	5'- GTCCAGGTCATTCACATCAAGTTC -3'
mTFA	NM_031326.2	F:	5'- CCCACAGAGAACAGAAACAG -3'
		R:	5′- CCCTGGAAGCTTTCAGATACG -3
GAPDH	NM_017008.4	F:	5'- AATTGCAGCCATGTGGAGG -3'
		R:	5'-AGTTGTCATGGATGACCTTGG-3'

2.12. Preparation for Histopathological Examination

Histopathological examination of the liver tissues was carried out according to Drury and Wallington (1980).

2.13. Statistical Analysis

SPSS software package version 18.0 was used to analyze data (SPSS Chicago, IL, USA) (Hagen, 2002). The data were expressed mean ± SD and analyzed using ANOVA to compare between different groups.

3- Results and Discussion

3.1. Brain and plasma serotonin, dopamine and norepinephrine levels and acetylcholine esterase activity

Rats treated with *Citrillus colocynthis* extract (CCE) alone showed no significant effects on the brain and plasma dopamine, serotonin, norepinephrine, and AChE. On the other hand, rats treated with carboplatin/thalidomide significantly caused marked decline in the brain and plasma dopamine, serotonin, norepinephrine, and caused significant activation of AChE compared to control and CCE groups ($P<0.05$). The co-administration of CCE with carboplatin/thalidomide significantly ameliorated the brain and plasma levels of neurotransmitters and the activity of AChE compared to control and CCE & carboplatin and thalidomide groups ($P<0.05$). The level of norepinephrine was completely normalized with CCE co-supplement. However, the levels of dopamine, serotonin, and the activity of AChE significantly differed from control values (Figures 1 and 2).

Amptoulach and Tsavaris (2011) reported that the most significant dose-limiting issue associated with platinum-based chemotherapeutic drugs is peripheral neurotoxicity. Also, neurotoxicity is one of the main drawbacks of thalidomide treatment alone or in combination with carboplatin (Miller *et al.*, 2006). Thalidomide-associated neurotoxic effects appeared in 40% of patients receiving thalidomide in a dose-dependent manner. This neurotoxicity is due to damage of the dorsal root ganglia (Beijers *et al.*, 2012).

3.2. Molecular effect

The obtained data obviously indicated that *Citrullus colocynthis* extract alone showed no significant effects on NF-κB compared with control. In contrast, rats exposed to chemotherapeutic agents (thalidomide and carboplatin) showed significant ($P<0.05\%$) elevation in NF-κB compared to control. The CCE co-treatment with thalidomide and carboplatin significantly protected inflammation mediator parameter from the deleterious impacts of chemotherapy drugs compared to chemotherapy treated group. However, the ameliorative effect of CCE on the previous parameter did not completely reach the control levels (Figure 3).

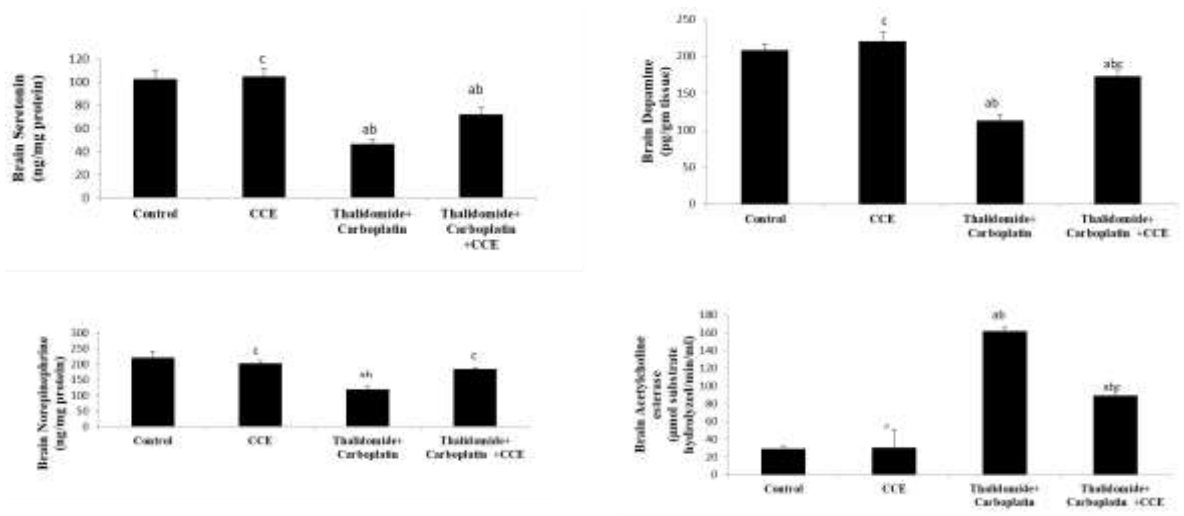

Figure 1: Brain serotonin, dopamine and norepinephrine levels and acetylcholine esterase (AChE) activity of male rats treated with *Citrullus colocynthis* extract (CCE), thalidomide + carboplatin and their combination (Carboplatin + Thalidomide + CCE).

Data presented as Mean ± SE

[a] Significantly differ Vs. control ($P<0.05$)

[b] Significantly differ Vs. CCE ($P<0.05$)

[c] Significantly differ Vs. carboplatin + thalidomide ($P<0.05$)

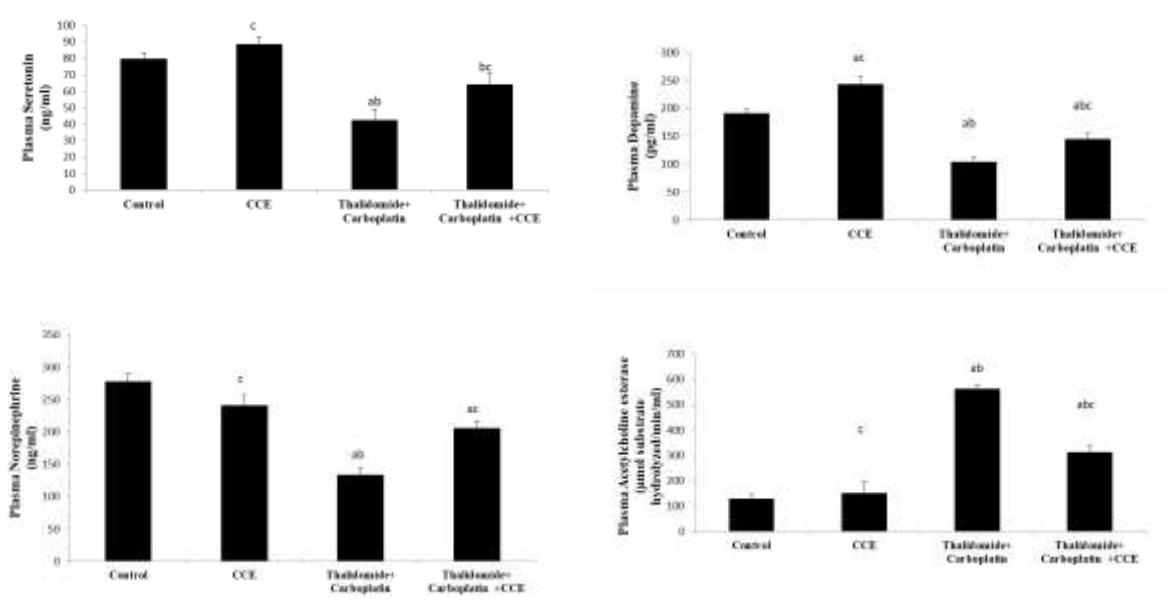

Figure 2: Plasma serotonin, dopamine and norepinephrine levels and acetylcholine esterase (AChE) activity of male rats treated with *Citrullus colocynthis* extract (CCE), thalidomide + carboplatin and their combination (Carboplatin + Thalidomide +CCE).

Data presented as Mean ± SE

[a] Significantly differ Vs. control (P<0.05)

[b] Significantly differ Vs. CCE (P<0.05)

[c] Significantly differ Vs. carboplatin + thalidomide (P<0.05)

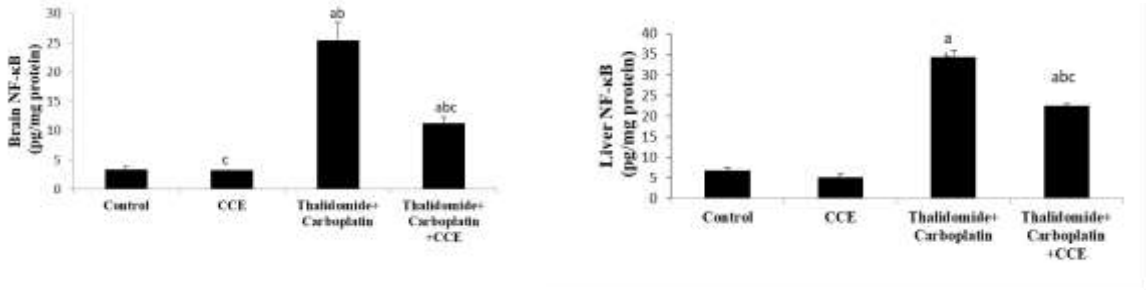

Figure 3. Changes in brain and liver inflammation mediator nuclear factor kappa B (NF-κB) of male rats treated with *Citrullus colocynthis* extract (CCE), thalidomide + carboplatin and their combination (Carboplatin + Thalidomide + CCE).

Data presented as Mean ± SE

[a] Significantly differ Vs. control (P<0.05)

[b] Significantly differ Vs. CCE (P<0.05)

[c] Significantly differ Vs. carboplatin + thalidomide (P<0.05)

The present data clearly indicated that *Citrullus colocynthis* extract alone showed no significant effects on caspase-3 compared with control. On the other hand, rats exposed to chemotherapeutic agents (thalidomide and carboplatin) showed significant (P<0.05%) elevation in caspase-3 compared to control and CCE groups. The CCE co-supplementation with thalidomide and carboplatin significantly protected apoptotic marker via decreasing the level of caspase-3 compared to chemotherapy treated group (Figure 4). However, the ameliorative effect of CCE on the previous parameters did not completely reach the control values.

The results of the present study showed marked activation in the activity of caspase-3 in the both brain and liver tissues of rats treated with carboplatin/thalidomide. Caspase-3 is the prominent apoptotic marker that indicate the irreversible execution phase of the apoptotic pathway. It was documented that lenalidomide (*thalidomide analog*) induces apoptosis and inhibits angiogenesis *via* caspase-3 and VEGF pathway (QU *et al*., 2016). Also, it was evidenced that, carboplatin alone or in combination with other chemotherapeutic drugs significantly induce the activity of caspase-3 (Gregoraszczuk *et al*., 2015). Shen *et al*. (2018) demonstrated a pro-apoptotic effect of carboplatin in a dose and time-dependent manner.

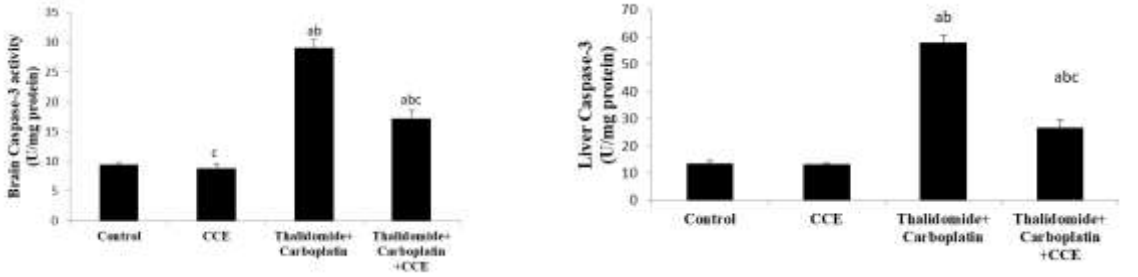

Figure 4: Changes in brain and liver activity of apoptotic marker cysteine aspartic acid specific protease (Caspase-3) of male rats treated with *Citrullus colocynthis* extract (CCE), thalidomide + carboplatin and their combination (Thalidomide+Carboplatin+CCE).

Data presented as Mean ± SE

[a] Significantly differ Vs. control (P<0.05)

[b] Significantly differ Vs. CCE (P<0.05)

[c] Significantly differ Vs. thalidomide + carboplatin (P<0.05)

The data indicated that *Citrullus colocynthis* extract alone showed significant increase in PGC-1a and mtTFA compared to control. In contrast, rats exposed to chemotherapeutic agents (thalidomide and carboplatin) showed significant (P<0.05%) reduction in PGC-1a and mtTFA compared to control and CCE &combination drugs group. The CCE co-treatment with thalidomide and carboplatin significantly protected gene expression parameters from the damaging impacts of chemotherapeutic drugs compared to chemotherapy treated group (Figure 5). However, the ameliorative effect of CCE on the previous parameters did not completely reach the control levels. At the level of gene expression, carboplatin/thalidomide significantly suppressed the brain and hepatic expression of peroxisome PGC-1 α and mitochondrial transcription factor A (mtTFA), both genes involved in the regulation of mitochondrial biogenesis. Mitochondria are the eukaryotic cells, powerhouses, it generates more than 90% of the energy required by the cell in the form of ATP which maintains cell viability (Teodoro *et al*., 2013). So, any disruption of mitochondrial homeostasis would be a key event in a wide variety of diseases and toxicological effects, and in even normal events such as aging (Meyer *et al*., 2013). The brain and liver are highly metabolic tissues and have intense demand for mitochondria (Wang *et al*., 2009). In order to meet the physiological needs of neuronal cells and hepatocytes, mitochondrial hemoostasis is maintainrd in which mitochondrial biogenesis plays an important role. The factors regulating mitochondrial biogenesis include mtTFA, which drives transcription and replication of mtDNA. The expression of mtTFA is regulated by PGC-1α, the master regulator of mitochondrial biogenesis (Chaturvedi and Beal, 2013). The decreased expression of PGC-1α and mtTFA in brain and liver tissues of rats treated with carboplatin/thalidomide reflects mitochondrial dysfunction through decreased mitochondrial biogenesis and mtDNA replication and transcription. This leads to the accumulation of electrons in the electron

transport chain complexes that can escape and directly react with oxygen to form the superoxide anion radical and induce oxidative stress.

The relation between the gene expression of nuclear transcription factors involved in mitochondrial biogenesis and function (mtTFA and PGC-1α) and neurotoxicity and hepatotoxicity of carboplatin/thalidomide was provided *via* theses data.

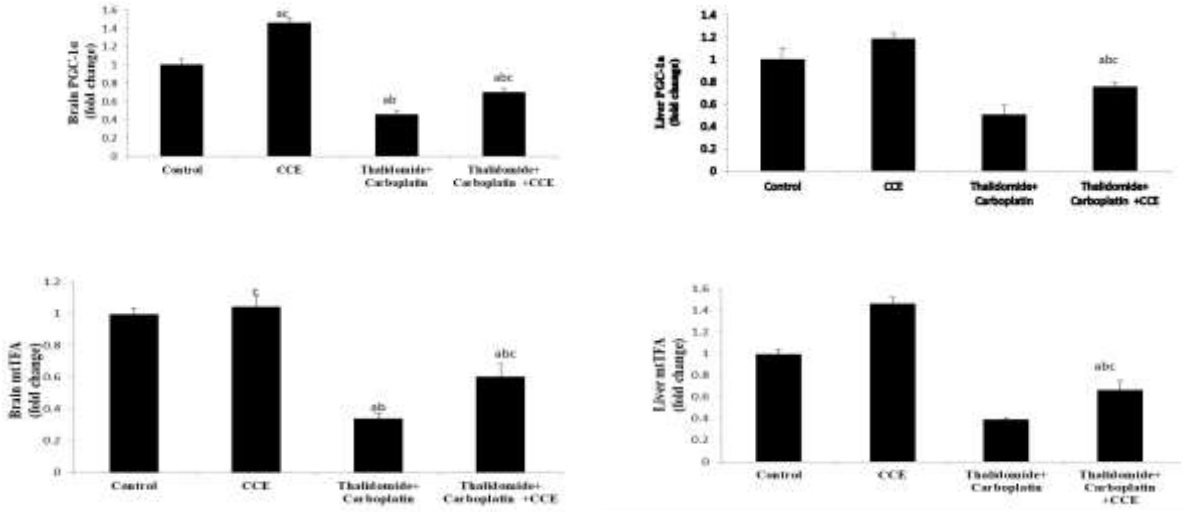

Figure 5: Changes in gene expression of brain and liver proliferator-activated receptor-gamma coactivator-1 (PGC-1a) and mitochondrial transcription factor A (mtTFA) of male rats treated with *Citrullus colocynthis* extract (CCE), thalidomide + carboplatin and their combination (Carboplatin +Thalidomide +CCE).

Data presented as Mean ± SE

[a] Significantly differ Vs. control (P<0.05)

[b] Significantly differ Vs. CCE (P<0.05)

[c] Significantly differ Vs. thalidomide + carboplatin (P<0.05)

The data indicated that *Citrullus colocynthis* extract alone showed no significant effects on TBARS and 8-OH-dG compared with control. On the other hand, rats exposed to tested chemotherapeutic agents showed significant (P<0.05%) elevation in TBARS and 8-OH-dG compared to control and CCE groups. The CCE co-supplementation with thalidomide and carboplatin significantly protected oxidative damage of both organs *via* decreasing the levels of TBARS and 8-OH-dG compared to control, CCE and chemotherapy treated groups (Figures 6 and 7). However, the ameliorative effect of CCE on the previous parameters did not completely reach the control values.

At the DNA level, carboplatin/thalidomide -induced oxidative stress caused a significant elevation in the oxidative DNA marker; 8-hydroxy-deoxyguanosine (8-OH-dG). ROS are extremely reactive with a number of biological substances, one of the most important of which is DNA (Mangerich *et al*., 2012). 8-OG-dG the prominent forms of oxidatively generated DNA base modifications and is thought to be a sensitive indicator of oxidative DNA damage (Cooke *et al*., 2003). If the repair mechanism did not remove 8-OH-dG formed in DNA, this may cause guanine (G) to thymine (T) substitution upon replication, alternatively, 8-OH-dG (in the nucleotide pool) may be miss incorporated opposite adenine (A) producing A-C substitution (Cheng *et al*., 1992). These mutagenic potentials of 8-OH-dG formation may indicate the hazards of carboplatin/thalidomide -induced genotoxicity.

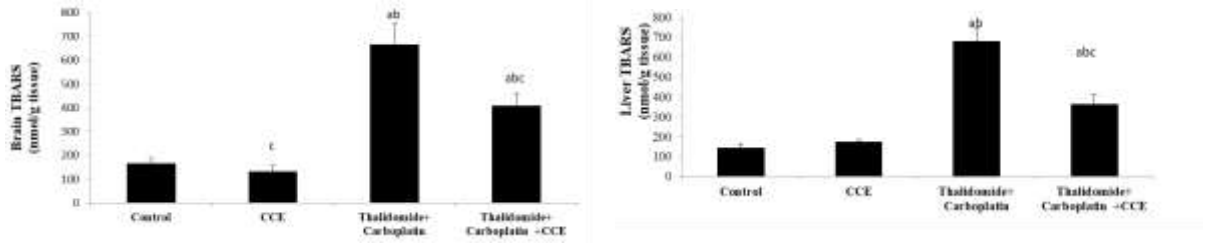

Figure 6: Changes in brain and liver thiobarbituric acid-reactive substances (TBARS) of male rats treated with *Citrullus colocynthis* extract (CCE), thalidomide + carboplatin and their combination (Carboplatin + Thalidomide +CCE).

Data presented as Mean ± SE

[a] Significantly differ Vs. control (P<0.05)

[b] Significantly differ Vs. CCE (P<0.05)

[c] Significantly differ Vs. thalidomide + carboplatin (P<0.05)

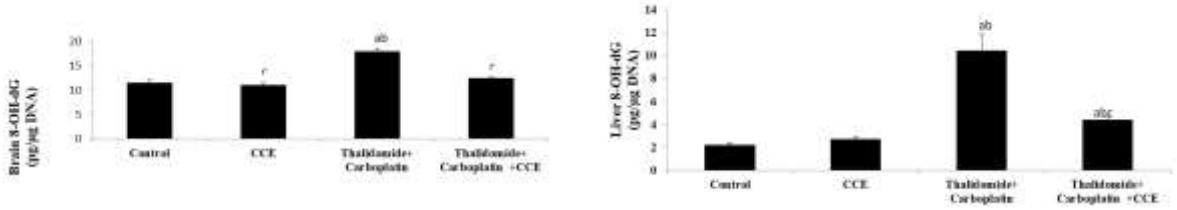

Figure 7: Changes in brain and liver 8-hydroxydeoxyguanosine (8-OH-dG) of male rats treated with *Citrullus colocynthis* extract (CCE), thalidomide + carboplatin and their combination (Carboplatin +Thalidomide +CCE).

Data presented as Mean ± SE

a Significantly differ Vs. control (P<0.05)

b Significantly differ Vs. CCE (P<0.05)

c Significantly differ Vs. thalidomide + carboplatin (P<0.05)

Data of brain and liver GSH and NRF2 showed that rats treated with *Citrullus colocynthis* extract alone caused significant effect on brain whereas did not cause any significant effects on theses parameters in liver compared with rats in the control group. In contrast, rats exposed to chemotherapeutic agents (thalidomide and carboplatin) showed significant (P<0.05%) reduction in GSH and NRF2 compared to control and CCE groups. The CCE co-treatment with thalidomide and carboplatin significantly protected antioxidant parameters *via* increasing the levels of GSH and NRF2 compared to chemotherapy treated group (Figures 8 and 9). However, the ameliorative effect of CCE on the previous parameters did not completely reach the control levels. The toxicity of carboplatin can be explained through various mechanisms including induction of oxidative stress through enhancing free radicals production and/or decreasing antioxidant defense mechanism (Silici *et al*., 2011), induction of pro-inflammatory pathways (Hussein *et al*., 2012), and impairment of mitochondrial functions (Wu *et a*l., 1999). *In vivo* interaction of free radicals with antioxidant system could induce a threshold for cancer only at concentrations exceeding the capacity of antioxidant defense mechanisms to control and eliminate resultant oxidative damage (Boreiko and Rossman, 2020).

The main cellular components involved in the regulation of antioxidant response are; nuclear factor erythroid 2-related factor 2 (NRF2), and antioxidant response elements (ARE). NRF2-ARE is a major signaling pathway that regulates the battery of cytoprotective proteins at the transcriptional level. NRF2-ARE contains various activation mechanisms for maintaining cellular redox balance and metabolism, in addition to inducing cytoprotective proteins

(Stewart *et al*., 2011). Proteins involved in drug metabolism, antioxidant response, and proteasomal degradation are all targets of NRF2 transcription (Baird and Dinkova, 2011). NRF2 suppression is desired in cancer, where NRF2 offers a survival advantage to malignant tumours (Robledinos *et al*., 2019). The inhibitory effect of carboplatin/thalidomide may be a therapeutic effect against cancer, however, in the normal tissues, the low level of NRF2 impairs the expression of many antioxidant proteins and enzymes including; thioredoxin, glutathione peroxidase, glutathione reductase and also the drug-metabolizing enzymes. All of these exaggerate oxidative stress and pro-inflammation leading to enhanced lipid peroxidation as indicated by the elevated level of TBARS.

Regarding the inflammatory status, carboplatin/thalidomide exposed rats showed marked significant elevation in the levels of inflammatory cytokines nuclear factor-κB (NF-κB). NRF2 is known to have an anti-inflammatory role through inhibition of the NF-κB pathway (Wu *et al*., 2019). So, the lower level of NRF2 induced by carboplatin/thalidomide together with oxidative stress may explain the increased levels of brain NF-κB.

Figure 8: Changes in brain and liver antioxidant reduced glutathione levels (GSH) of male rats treated with *Citrullus colocynthis* extract (CCE), thalidomide + carboplatin and their combination (Carboplatin +Thalidomide +CCE).

Data presented as Mean ± SE

[a] Significantly differ Vs. control (P<0.05)

[b] Significantly differ Vs. CCE (P<0.05)

[c] Significantly differ Vs. thalidomide + carboplatin (P<0.05)

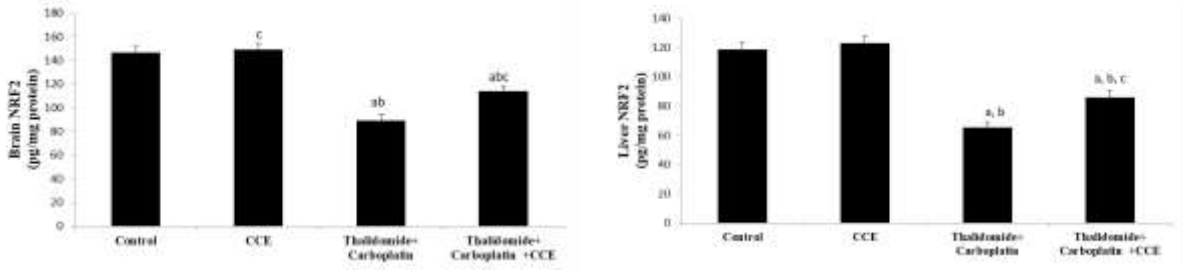

Figure 9: Changes in brain and liver nuclear factor erythroid-2-related factor 2 (NRF2) of male rats treated with *Citrullus colocynthis* extract (CCE), thalidomide + carboplatin and their combination (Carboplatin +Thalidomide +CCE).

Data presented as Mean ± SE

[a] Significantly differ Vs. control (P<0.05)

[b] Significantly differ Vs. CCE (P<0.05)

[c] Significantly differ Vs. thalidomide + carboplatin (P<0.05)

3.3. Biochemical parameters

The data clearly indicated that *Citrullus colocynthis* extract alone showed no significant effects on AST, ALT, ACP, ALP, total protein and albumin compared with control. On the other hand, rats exposed to chemotherapeutic agents (thalidomide and carboplatin) showed significant (P<0.05%) elevation in plasma AST, ALT, ACP and ALP. While, caused significant reduction in plasma total protein and albumin compared to control

and CCE groups. The CCE co-supplementation with chemotherapeutic agents significantly protected liver function enzymes and proteins from the deleterious effects of chemotherapeutic drugs compared to chemotherapy treated **(Table 2)**. However, the ameliorative effect of CCE on the previous parameters did not completely reach the control values. Al-Gaithi *et al*. (2004) evaluated the effect of CC aqueous seed extract on biochemical parameters in diabetic rats. They found significant decrease in plasma AST activity. Likewise, Alabadi and AL-Ali (2010) found significant decrease in serum ALT and ALP levels in diabetic rats treated with 300mg/kg aqueous fruit extract of CC. The electron microscopic study of Khalil *et al*. (2010) documented ameliorative effects of CC against hepatic damage of diabetic rats. Also, Ezzat-Ali Esmail (2012) revealed a protective effect of the CC fruits seeds extract in preventing the onset of the fatty liver syndrome as well as diabetes mellitus type 2 induced by exposure to high-fat diet and this action was dose dependent.

Table 2: Changes in activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP), alkaline phosphatase (ALP), total protein and albumin in plasma of male rats treated with *Citrullus colocynthis* extract, thalidomide plus carboplatin and their combination (Means ± SE)

Parameters	Control	CCE	T + C	CCE+ T+C
AST (U/L)	54.25 ± 4	47.25 ± 3.96	187 ± 13.17 [a, b]	123 ± 12.81 [a, b, c]
ALT (U/L)	30.25 ± 3.63	34.50 ± 4.33	112.50 ± 14.73 [a, b]	71.25 ± 6.42 [a, b, c]
ACP (U/ L)	4.07 ± 0.70	4.07 ± 0.70	11.80 ± 1.21 [a, b]	6.55 ± 1.67 [a, b, c]
ALP(U/L)	38.25 ± 5.70	42.50 ± 8.61	102.75 ± 9.56 [a, b]	69.75 ± 5.62 [a, b, c]
Total protein(g/dl)	7.80 ± 0.11	7.82 ± 0.20	6.67 ± 0.26 [a, b]	7.57 ± 0.28 [c]
Albumin (g/dl)	4.17 ± 0.11	4.00 ± 0.21	2.67 ± 0.21 [a, b]	3.35 ± 0.19 [a, b, c]

Data presented as Mean +SE

[a] Significantly differ Vs. control (P<0.05)

[b] Significantly differ Vs. CCE (P<0.05)

[c] Significantly differ Vs. carboplatin + thalidomide (P<0.05)

3.4. Histopathological findings

Paraffin sections were stained with hematoxylin and eosin for morphological changes in rat tissues for normal and experimental groups. Hepatocytes cells (H) with round dark nuclei and homogenous cytoplasm. They lose their radiating and mild dilation of sinusoids (S) was seen (Figure 10). *Citrullus colocynthis* extract of rat liver, showed mild dilation of portal tract (PT) with mild dilated bile duct (BD) and two central vein (CV) with absence of fibrotic cells. Hepatocytes cells (H) with round nuclei with homogenous cytoplasm, mild dilation of sinusoids (S) (Figure 11). Thalidomide and carboplatin rat liver, showed four adjusting marked dilation of portal tract (PT) surrounded by infiltrating lymphocytes (IF) and fibrotic cells (F). Crowded hepatocytes cells (H) with round dark nuclei, few hepatocytes with homogenous cytoplasm necrotic cell (NC) surrounded the marked dilation of central vein (CV), mild dilation of sinusoids (S) (Figure 12). Thalidomide, carboplatin + CCE treatment rat liver, showed three adjusting marked dilation of portal tract (PT) with mild dilated bile duct (BD) and associated by two central veins (CV). Hepatocytes cells (H) with round dark nuclei with homogenous cytoplasm surrounded the marked dilation of central vein and portal tract, mild dilation of sinusoids (S). (H&E stains X100 Mag) (Figure 13).

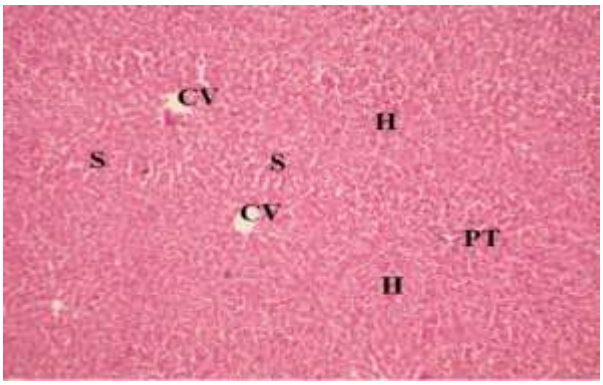

Figure 10: Paraffin section photomicrograph of control rat liver illustrating hepatocytes

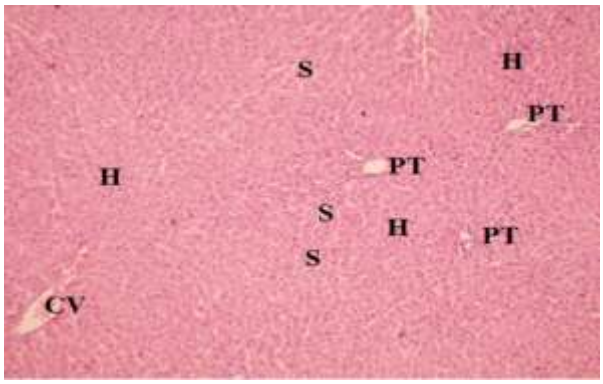

Figure 11: Paraffin section photomicrograph of Citrullus colocynthis extract of rat liver

cells (H), sinusoids (S), central vein (CV) and portal tract (PT). (H&E stains X100 Mag).

illustrating hepatocytes cells (H), sinusoids (S), portal tract (PT) and central vein (CV). (H&E stains X100 Mag).

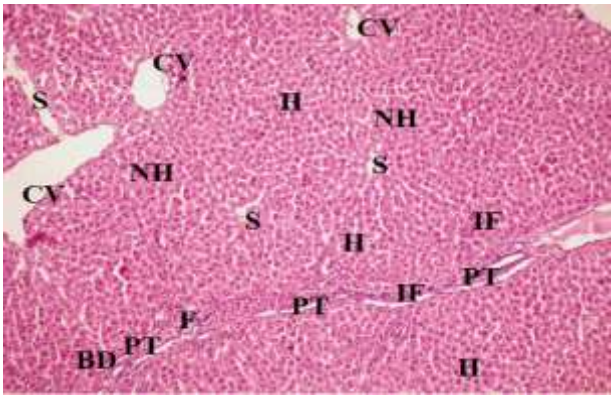

Figure 12: Paraffin section photomicrograph of thalidomide and carboplatin rat liver illustrating hepatocytes cells (H), sinusoids (S), portal tract (PT), infiltrating lymphocytes (IF), fibrotic cells (F), necrotic cell (NC), bile duct (BD), necrotic hepatocytes (NH) and central vein (CV). (H&E stains X100 Mag).

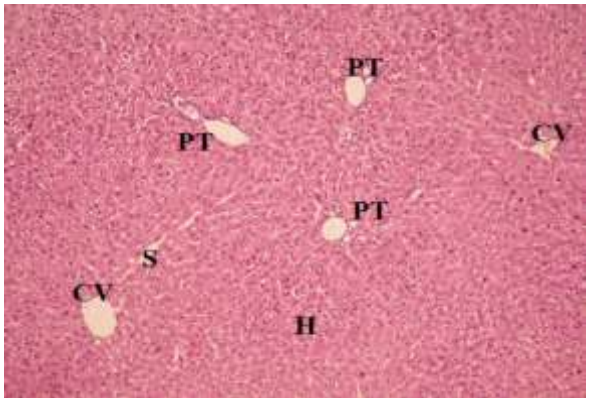

Figure 13: Paraffin section photomicrograph of carboplatin + thalidomide + CCE treatment rat liver illustrating hepatocytes cells (H), sinusoids (S), portal tract (PT) and central veins (CV). (H&E stains X100 Mag).

Collectively, the results of the present study confirm the neurotoxicty and hepatotoxicty of carboplatin/thalidomide which was mediated through suppressing the antioxidant systems, mitochondrial biogenesis, and induction of inflammation. Given the fact of wide use of carboplatin/thalidomide in the field of medical oncology, the search for protective interventions against their toxicity is of great medical importance. In this study, we explored the protective effects of *Citrullus colocynthis* extracts (CCE). The results of the present study indicated the protective effects of CCE against the carboplatin/thalidomide -induced neurotoxicity and hepatotoxicty. CCE is a known natural product that is widely used for human dietary use with great anti-oxidants and anti-inflammatory effects (Marzouk *et al*., 2010). In agreement with the present study, the antioxidant effects of CCE were confirmed in many studies that confirmed that *CCE* is very rich in antioxidants (e.g. polyphenol and plant sterol) (Sebbagh *et al.*, 2009). The fruit extracts have excellent antioxidant potential (Kumar *et al*., 2008).

The results of the present study indicated that CCE significantly ameliorated the deteriorations of most studied parameters. At the histological level, the CCE co-supplementation with carboplatin/thalidomide reserved a moderate degree of improvement in the hepatocytes damage with only a minimal vacuolization in hepatocytes and moderate congestion of the central veins compared to rats treated with thalidomide and carboplatin. The observed improvements induced by CCE extract were associated with significant amelioration of the plasma and brain neurotransmitters (dopamine, serotonin, and norepinephrine) and AChE activity compared to the carboplatin and thalidomide-treated rats. It was documented that CCE exhibited metal chelation and reduction capability and effective inhibition of AChE (Shadat *et al*., 2015). Therefore, may help in preventing or alleviating patients suffering neurodegenerative diseases. Mehrzadi *et al*. (2016) found that *Citrullus colocynthis* had a strong anticonvulsant impact in mice with pentylenetetrazole-induced seizures, and that these effects could be linked to its opioid system actions. Also, it was documented that, CCE possesses significant anti-inflammatory and anti-nociceptive activities in animal models, which were probably mediated by opioid receptors and the suppression of pro-inflammatory cytokines (Pashmforosh *et al*., 2018). Chen *et al*. (2019) validated that CCE had a promising neuro-protective effect against Parkinson's disease, through reducing oxidative stress, enhancing antioxidant enzymes and inhibiting autophagic cell death. Barth *et al*. (2002) found that CC extract in doses of 100 µg/ml reduced lipid peroxidation and ROS production induced by CCl_4.

The results of our study demonstrated the boosting effects of CCE on the antioxidant status of brain and hepatic tissues through enhancing the levels of GSH and NRF2. On the other hand, CCE significantly declined the tissues levels of MDA and 8-OH-dG, the markers of lipid and DNA oxidative damage, respectively. Regarding the

cytokine levels, the co-supplementation with CCE significantly declined the elevated levels of NF-κB in both brain and hepatic tissues that was induced by carboplatin and thalidomide. NRF2 activation by CCE may inhibit NF-κB pathway mainly by elevating antioxidant defenses which neutralize ROS and detoxifying chemicals (Soares, 2004). It was documented that CC exhibited anti-inflammatory activity through down-regulating the levels of inflammatory cytokines (Wasfi *et al*. 1995). All these effects resulted in significant decline in both brain and the hepatic activity of the apoptotic marker; caspase-3.

At the molecular level, the present study demonstrated that co-supplementation of carboplatin/thalidomide-treated rats with CCE improved the brain and liver expression of PGC-1α and mtTFA genes which may imply restoring mitochondrial biogenesis and function.

Miao *et al*. (2012) investigated the *in vivo* hepatoprotective activities of this plant using the fruit containing seeds, which contain many cucurbitacins, such as dihydro cucurbitacin E, cucurbitacin E, dihydro-epi-iso-cucurbitacin D, dihydro iso-cucurbitacin B-25-acetate, and cucurbitacin E-2-O-β-D-glucopyranoside. Plants containing cucurbitacins have been known for their antipyretic, analgesic, anti-inflammatory, antimicrobial, antitumor (Lee *et al*., 2011), and liver protective activities (Bartalis and Halaweish, 2011) in folk medicine. This indicated that, the liver protective effect may be dependent on the cucurbitacins of *c. colocynthis* (Yang *et al*., 2011).

Conclusion

From the above discussion it was clear that, the exposure to carboplatin/thalidomide induce neurotoxicity and hepatotoxicity at different levels including, histological structure, neurotransmitters, redox status, DNA damage, cytokine production, apoptosis and gene expression of genes regulating mitochondrial biogenesis; PGC-1α and mtTFA. This study provided solid evidences of the protective effects of CC extract against toxicity induced by carboplatin and thalidomide. CC extract is safe, efficient and promising therapeutic agent that can be effectively employed as natural therapeutic antioxidant for a wide range of applications in the field of food and medicine.

Disclosure

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. There is no Conflict of Interest

Ethical Approval and consent to participate

This research was approved by the local ethical guidelines of Institutional Animal Care & Use Committee (IACUC), Alexandria University, Egypt (AU14-21026-2-4).

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