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VINCRISTINE-INDUCED NEUROTOXICITY IN RATS MEDIATED BY UPREGULATION OF INOS, IBA1, NESTIN, PARP AND CASPASE 3: AMELIORATIVE EFFECT OF ERYTHROPOIETIN AND THYMOQUINONE

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Received: 16 July 2023; **Accepted:** 20 August 2023

ABSTRACT

Vincristine (VCR) is an effective anticancer medication, although it has neurotoxic side effects. Erythropoietin (EPO) is the main regulator of erythropoiesis. Thymoquinone (TQ) protects brain cells from oxidative stress that causes neurodegenerative disorders such as Alzheimer's and Parkinson's. This study aims to investigate the VCR toxicity on the cerebrum as well as the possible neuroprotective effects of TQ and EPO against VCR toxicity in a rat model. An intraperitoneal injection of VCR (150 µg/kg) 3 times a week for 5 weeks caused marked histopathological changes in the brain such as neuronal degeneration with aggregations of glial cells around the degenerated neurons (satellitosis), congestion of blood vessels and severe demyelination in the white matter of the cerebrum. VCR considerably increased nestin, iBA1 and iNOS expression, while synaptophysin expression decreased. It also caused upregulation of caspase 3 and PARP expression, resulting in hemorrhage, demyelination, and neuronal degeneration. Treatment of rats with TQ or EPO either alone or in combination improved histopathological changes through down-regulation of nestin, iBA1, iNOS, caspase 3 and PARP. It was concluded that EPO and TQ ameliorate the neurotoxic effect of VCR on the cerebrum, however, a synergetic effect was evident when TQ and EPO were combined.

Keywords: Vincristine, brain, erythropoietin, thymoquinone, apoptosis, demyelination.

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INTRODUCTION

Many medicinal plants contain alkaloids, including in doles (Singh and Singh, 2018), which can regulate cell death by targeting related signaling pathways (Song *et al.,* 2021). Vincristine (VCR) is an indole alkaloid natural product that has FDA approval as an antitumor medication and is used as the first line of cancer treatment (Patridge *et al.,* 2016). These agents are however limited in their use due to their significant side effects on the nervous system and bone marrow (Jordan, 2002). Indole alkaloids target cancer cells by different mechanisms, including autophagic cell death and apoptosis (Qin *et al.,* 2022).

Apoptosis is an important target in cancer treatment and it has become an important component of the development and discovery of novel anticancer (Wang *et al.,* 2018). The treatment consists of mollifying chemotherapy and corresponds to the treatment of extensive-stage small-cell lung cancer (Olsen *et al.,* 2012). PARP-1 is a nuclear protein and has a wide range of pathological and physiological functions. PARP-1 plays important roles in astrocyte regulation, microglial function, and long-term memory and aging (Chaitanya *et al.,* 2010). PARP-1 is cleaved by caspases which are considered a mark of apoptosis (Kaufmann *et al.,* 1993). Therefore, it was hypothesized that the use of both caspase and PARP inhibitors could be a crucial therapeutic in disorders where both necrosis and apoptosis occur (Los *et al.,* 2002).

Erythropoietin (EPO) is a glycoprotein consisting of 166 amino acids produced by the kidney and acts as both a hematopoietic growth factor and a peptide hormone that stimulates bone marrow erythropoiesis and has several roles outside the bone marrow (Lund *et al.,* 2014). Recombinant human EPO could pass through the blood-brain barrier (Brines et al., 2000), so investigators are concerned with studying its role in the nervous system. The EPO receptor (EPOR) is expressed in several tissues, including endothelial cells and astrocytes in the CNS, that can produce and secrete EPO (Messé *et al.,* 2013). Furthermore, EPO has been found to be a regenerative or protective hormone that can enhance neurological illnesses (Nekoui & Blaise, 2017).

Thymoquinone (TQ), which is the phytochemical bioactive constituent of *Nigella sativa* seeds, has high anti-inflammatory, anticancer, antioxidant, and neuroprotective properties (Kooti *et al.,* 2016). TQ is a promising medication for reducing chemotherapy toxicity (AbuKhader, 2013). TQ showed anticancer properties since it prevents oxidative stress and inflammation, inhibits metastasis and angiogenesis, induces apoptosis, stimulates the expression of tumor suppressor genes and reduces the expression of tumor-promoting genes (Alhmied *et al.,* 2021).

This study aims to estimate the neuroprotective efficacy of TQ, EPO, and their combination against VCR-induced cerebral toxicity in rats.

MATERIALS AND METHODS

1. Ethics approval

The animal procedures were approved by MBRSI- Research Ethics Committee number IORG0010947-MB-21-6-A.

2. Animals

Fifty male albino rats (180±20g) were purchased from the Animal House Laboratory, Department of Pathology& Clinical Pathology, Faculty of Veterinary Medicine, Assiut University. All rats were housed and adapted for two weeks before the experiment at room temperature with a normal light/dark cycle with free access to food and water.

3. Chemicals

TQ (Sigma Aldrich, MO, USA), Vincristine sulfate (Hikma Pharmaceuticals, Giza, Egypt), and Human recombinant EPO (SEDICO, 6th October City, Egypt) were used. Rabbit polyclonal primary antibody against PARP was purchased from Invitrogen (Carlsbad, CA), β-actin and

cleaved caspase-3 were obtained from Abcam (Cambridge, UK), Iba-1 from Fujifilm Wako Chemicals (VA, USA), synaptophysin from SYSY (Goettingen, Germany), NOS-2/iNOS from Bioss (MA, USA). Mouse monoclonal antibody against nestin (10-c2) was purchased from Santa Cruz (TX, USA). Horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG was from Santa Cruz.

4. Experimental design

The rats were randomly divided into 5 groups(n=10): Group I (control group) only received saline; Group II (VCR group) was treated with VCR (150µg/kg, intraperitoneal (IP) 3 times weekly for the entire experiment (Ja'afer, Hamdan, & Mohammed, 2006); Group III (EPO group) received VCR + EPO (80µg/kg, IP) (Kassem, El-Din, & Yassin, 2011); Group IV (TQ group) received VCR + TQ (10mg/kg, oral)(Mehri *et al.,* 2014); and Group V (EPO+ TQ group) received VCR + EPO and TQ. All rats were observed for 5 weeks and then sacrificed. Brain specimens were collected for subsequent analyses (histopathology, immunohisto-chemistry, and western blot).

5. Histopathological examination

Tissue samples were collected, fixed in 10% neutral-buffered formalin, routinely processed, and embedded in paraffin wax. Paraffin-embedded tissues were then sectioned at 5 µm thickness and stained with hematoxylin and eosin (Suvarna, Layton, & Bancroft, 2018). The slides were then examined microscopically (Olympus CX31, Japan) and photographed (Olympus, Camedia C-5060, Japan). Histopathological scoring was performed based on the blood vessel congestion, hemorrhage, neuronal degeneration, and demyelination from three random sections.

6. Immunohistochemical examination

The paraffin sections were deparaffinized, rehydrated with graded ethyl alcohol, and washed (3 times, 5 min each) with PBS. Antigen retrieval was carried out by boiling the slides for 10 min in 1 mM sodium citrate buffer (pH 6). The endogenous peroxidase activity was quenched with 3% H_2O_2 for 25 min at 37°C, then the sections were washed with PBS and incubated with 10% normal goat serum in 0.2% Triton-X 100 /PBS at 37°C for 2 h to block nonspecific reactions. The sections were incubated with the primary antibodies overnight at 4°C, rinsed with PBS (3 times, 10 min), and then treated with the Ultra Tek HRP anti-polyvalent kit (Goat anti-mouse, rat, rabbit and Guinea pig IgG) as a secondary antibody which (ScyTek, USA). Visualization of the reactions was done with DAB for 5-10 min and counterstained with Harris hematoxylin (Attaai *et al.,* 2022).

7. Western blot analysis

Tissue homogenates were centrifuged at 1500 rpm for 5 min at room temperature, the pellets were washed with ice-cold PBS buffer (2 times), then the cells were lysed with cold RIPA buffer (5 mM EDTA, 50 mMTris-Cl [pH 7.6], 150 mM NaCl, 0.5% Triton-X-100, and 0.5% NP-40) containing 1 µg/mL aprotinin and leupeptin, and 0.5 mM PMSF. The lysates were centrifuged at 4°C for 10 min at 2500 rpm. Then the protein concentrations were determined by Bradford assay. 40 µg of the protein aliquots were electrophoresed on 10% SDS–PAGE gels and then transferred onto nitrocellulose membranes. Membrane blocking was done using 2% BSA and then probed with primary antibodies (anti-PARP, anti-cleaved-caspase3, and anti-β-actin, 1:1000) at 4°C overnight. Then membranes were incubated with the HRP-conjugated secondary antibody (1:10,000) for 1 h at room temperature. ECL substrate was used for detection. The immunoreactive bands were densitometrically calculated using Image J software (Fouad, Elsokkary, & Shakor, 2022).

8. Statistical analysis

Results are presented as means ± standard deviation (SD) of three independent replicates. One-way analysis of variance (ANOVA) followed by Newman-Keuls post-test (Keuls, 1952; Newman, 1939) was

used to evaluate the differences between the tested groups. The results were considered statistically significant at $p < 0.05$.

RESULTS

1. Histopathological evaluation

Histopathological analysis of the brain sections from the control group showed the normal morphological structure of neurons, blood vessels, and glial cells in the cerebrum (Fig 1a). In contrast, brain sections from group II (VCR group) showed neurodegeneration with aggregation of glial cells around the degenerated neurons (satellitosis) (Fig 1b), significant demyelination in white matter and congestion of blood vessels (Fig 1c) in the cerebrum.

However, the administration of EPO or TQ as single (group III or IV, respectively) or in combination (group V) showed ameliorative effects on the pathological changes caused by VCR in the cerebrum which appeared more or less normal (Fig 1d,e, f). All histopathological results of the brain in different groups were scored in Table 1.

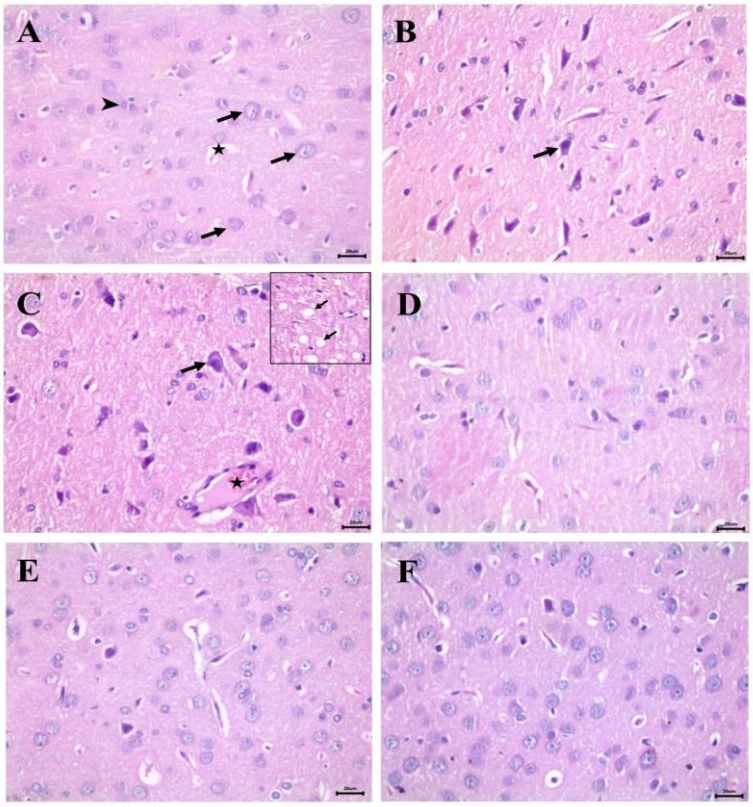

Fig.1: Photomicrograph of the cerebrum from different groups. A. The control group shows normal neurons (arrows), glial cells (arrowhead), and blood vessels (asterisk). B and C. VCR group shows neuronal degeneration with satellitosis (arrow, B), neuronal degeneration (arrow, C), congestion (asterisk, C), and severe demyelination (inset, C). D. EPO group and E. TQ group show normal neurons with mild neuronal degeneration. F. EPO+TQ group shows normal neurons. H&E stain, scale bars = 20 μM.

Table 1: Scoring of histopathological results of the brain tissue in various groups.

Histopathological findings	Control	VCR	VCR+EPO	VCR+ TQ	VCR+ EPO+TQ
Blood vessels Congestion	-ve	+++	++	+++	++
Hemorrhage	-ve	+++	+++	++	+++
Neuronal degeneration	-ve	+++	+	++	+
Demyelination	-ve	+++	+	++	+

Intensity scores:-ve=Not found,+=Mild, ++=Moderate,+++= severe

2. Immunohistochemical evaluation

Glial and neuronal progenitors were marked with the expression of nestin. The intensity of IBA1 was lower in control rats and was increased in VCR-treated rats and almost returned to the normal control by treating rats with EPO or TQ or their combination (Fig 2). Nestin immunoreactivity was increased in the cerebral cortex of rats treated with VCR, however, was decreased in the brain of rats treated with TQ or EPO their combination (Fig 3). Activation of microglia usually occurs as an initial response of the CNS to several pathological stimuli to employ a cytotoxic function by releasing NO, ROS, or inflammatory cytokines. So, the intensity of iNOS immunoreactivity in the cerebral cortex of rat brains treated with VCR was increased, however, it was decreased almost to the normal control by co-treatment with TQ or EPO or their combination (Fig 4). Immunoreactivity of the pre-synaptic protein synaptophysin, the main protein of the synaptic membrane that plays an important role as a channel in synaptic vesicle exocytosis, was decreased in the cerebral cortex of rat brain treated with VCR, however, was increased in rats-cotreated with EPO, TQ or their combination (Fig 5).

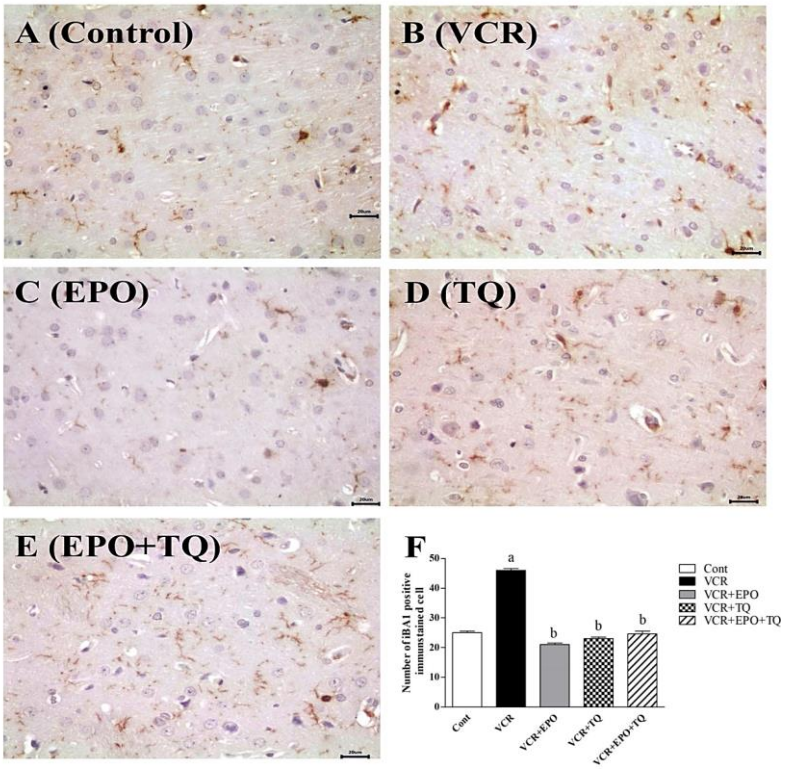

Fig. 2: Immunohistochemical iBA1 staining of cerebral cortex in rat brains. The intensity of the iBA1 was higher in VCR treated group compared with the control and co-treated rats with TQ or EPO or their combination.

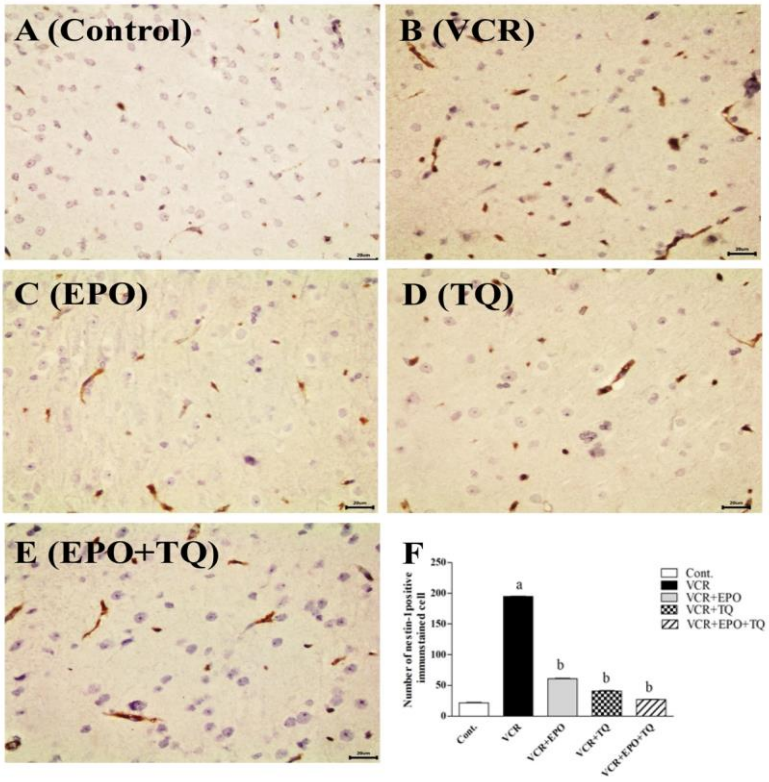

Fig. 3: Immunohistochemical nestin staining of cerebral cortex in rat brains showing that the intensity of the nestin was higher in the VCR-treated group compared with the control and co-treated rats with TQ or EPO or their combination.

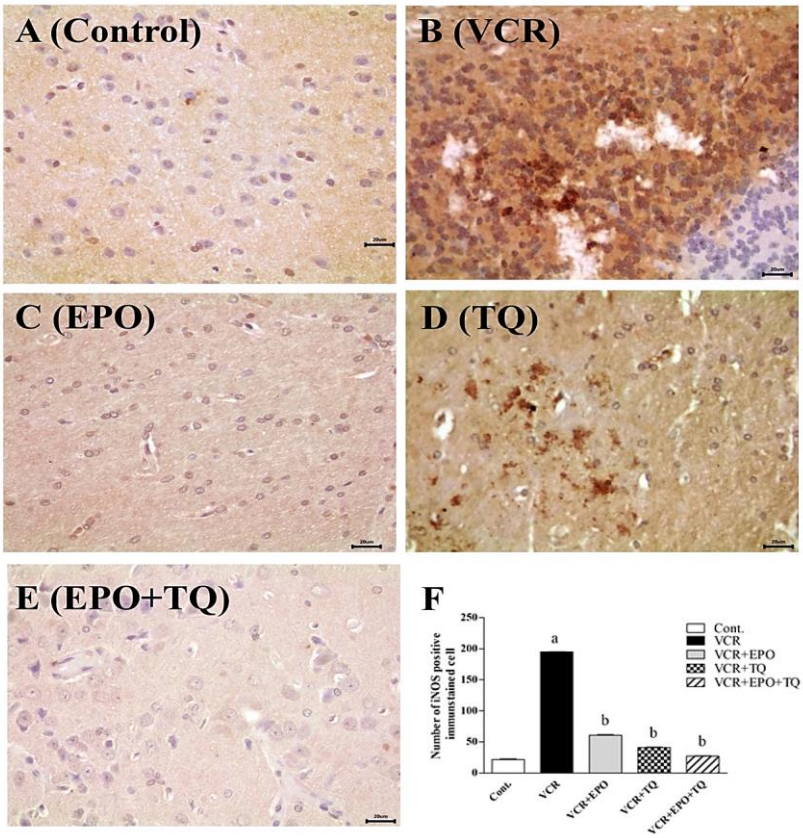

Fig.4: Immunohistochemical iNOS staining of cerebral cortex in rat brains. The iNOS intensity (arrows) was higher in VCR treated group compared with the control and co-treated rats with TQ or EPO or their combination.

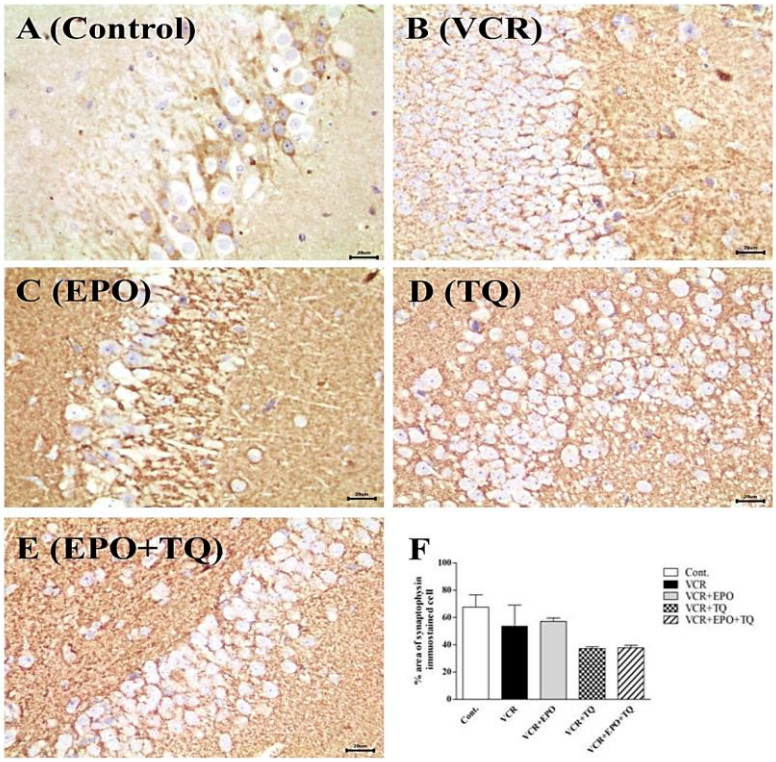

Fig.5: Immunohistochemical synaptophysin staining of cerebral cortex in rat brains. The synaptophysin intensity was lower in VCR treated group and co-treated rats with TQ or EPO or their combination compared with the control.

3. Effect of VCR and co-treatment with TQ or EPO or their combination on the caspase-3 and PARP-1 cleavage in the brain of rat

Although its role in DNA repair, over activation of PARP-1 in neuronal excitotoxicity caused induction of cell death. In the present experiment, as in Figs. 6 and 7, the western blot results showed an elevated level of caspase-3 and PARP-1 in the VCR-treated group compared to the control group. EPO or TQ or their combination with VCR showed a significant decrease in both caspase-3 and PARP-1 levels with the priority of the combination of TQ and EPO.

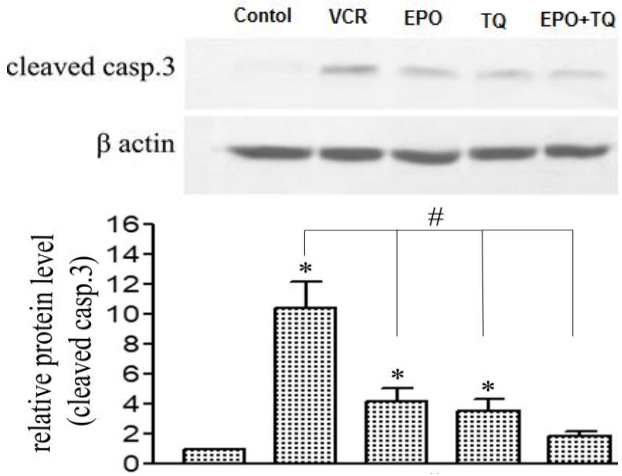

Fig. 6: Western blot results of the cleaved caspase-3 in the brain of control and different treated groups. ß-actin is taken as control. A: a representative Immunoblot of the cleaved caspase-3. B: The density

values were expressed in mean ± SEM (n=3) after normalization to the corresponding ß-actin bands. * and # P < 0.05 versus control and VCR groups; respectively.

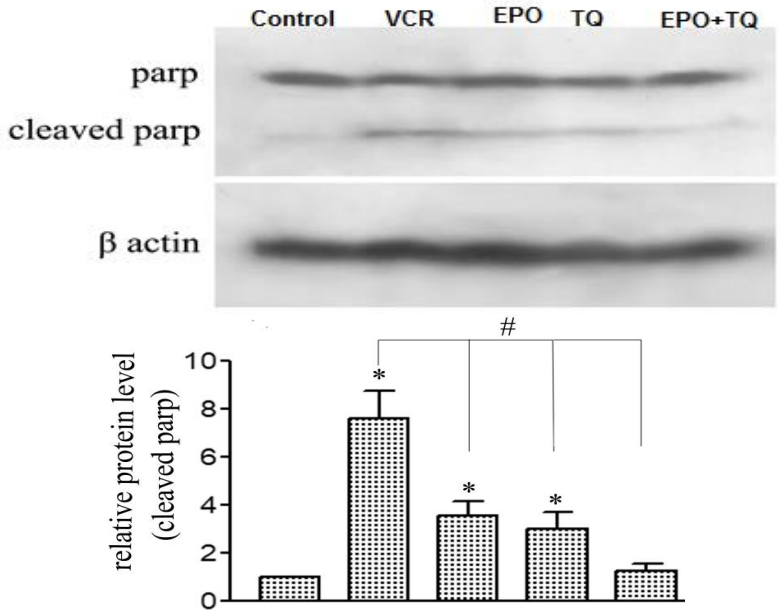

Fig. 7: Western blot results of PARP in the brain of control and different treated groups. ß-actin is taken as control. A: a representative Immunoblot of PARP and cleaved PARP. B: The density values were expressed in mean ± SEM (n=3) after normalization to the corresponding ß-actin bands. * and # P < 0.05 versus control and VCR groups; respectively.

DISCUSSION

The microtubules are an important component of the cytoskeleton that plays an important role in different eukaryotic cellular processes, such as cell division and growth (Desai & Mitchison, 1997). VCR as microtubule-targeting agents are an important class of anticancer drugs because of their capacity to interact with tubulins (Y.-M. Liu, Chen, Lee, & Liou, 2014; Mitchison, 2012). Inhibition of microtubule formation causes mitosis arrest in the metaphase, by restricting the formation of a mitotic spindle. Moreover, VCR overlaps with both nucleic acid and protein synthesis by blocking the use of glutamic acid (Martino *et al.*, 2018). In addition, VCR disrupts the active transport of proteins and other components within neurons (Carlson & Ocean, 2011). As shown in the present study, VCR-treated rats showed neuronal degeneration with aggregations of glial cells around the degenerated neurons with demyelination of white matter of the cerebrum. As a result of VCR binding to microtubules neurons die (Starobova & Vetter, 2017), because microtubules are necessary components of oligodendrocytes, which are responsible for myelination of nervous fibers (Lee & Hur, 2020). Moreover, VCR caused mitochondrial damage (Canta *et al.*, 2015) by modulating mitochondrial absorption and concentration of Ca^{2+} (Islam *et al.*, 2019), leading to increased exocytosis of neurotransmitters and activation of apoptosis (Marchi *et al.*, 2018).

In this study, histological improvement in the cerebrum of rats co-treated with either TQ, EPO or their combination was observed. rhEPO is known to have beneficial effects on non-motor symptoms associated with Parkinson's disease (Jang *et al.*, 2014), suggesting that it could be used as a new method of treating brain disorders (Merelli *et al.*, 2013). Also, TQ has been shown to protect brain tissue from oxidative stress induced by radiation (Ahlatci *et al.*, 2014) and efficiently attenuated Aβ1-42-induced neurotoxicity in cortical neurons (Alhebshi *et al.*, 2013). Therefore, TQ may be used to reduce the toxic effects of chemotherapeutic agents by inducing cell cycle arrest and the down-regulating pro-apoptotic genes (Darakhshan *et al.*, 2015), and initiating apoptosis via

activation of caspases-3 (Paramasivam *et al.,* 2012).

Neurodegenerative disorders are frequently caused by dysregulated apoptosis, which causes increased or decreased cell death. The caspase activation which is typically present as inactive zymogen forms is one of the most frequent signaling cascades contributing to apoptosis. When it is activated, caspases initiate cell death by removing and activating effector caspases that drive the apoptosis process (Yakovlev & Faden, 2001). The cleavage of PARP-1 by caspases is the hallmark of apoptosis (Kaufmann *et al.,* 1993). In this study, rats treated with VCR showed upregulation of cleavage caspase3 and cleavage PARP. The cleavage of PARP by caspase-3 has been involved in different neurological diseases e.g. Alzheimer's disease, cerebral ischemia, Parkinson's disease, multiple sclerosis, brain tumors, especially gliomas, and traumatic brain injury (Gilliams-Francis *et al.,* 2003; Lau *et al.,* 2006). In normal conditions, the PARP-1 primary function is to detect DNA damage and to repair it. However, cells with significant DNA damage have increased PARP-1 activity, leading to high NAD+ consumption. This activity, if left unchecked, will inevitably result in passive necrotic cell death (caused by long ATP depletion). Rapid cleavage and inactivation of PARP-1 by caspases prevent this process from occurring. However, insults that initiate necrosis cause PARP-1 overactivation which proceeds unchecked due to insufficient caspase activation (Aikin *et al.,* 2004; Los *et al.,* 2002).

However, rats co-treated with TQ, EPO or their combination showed a relative decrease in cleavage caspase 3 and cleavage PARP compared to the VCR-treated group. TQ-specific inhibitory action on cancer cells is associated with activated caspase upregulation (Ashour *et al.,* 2016). Caspase-3 reduction which ends up in the process of cell death, and PARP, one of the targets of caspase-3, supported the apoptosis-inhibiting effect of TQ in healthy tissue (Beker *et al.,* 2018). Ethanol promoted caspase-dependent cleavage of PARP-1. Administration of TQ decreases DNA damage and inhibits cell death caused by ethanol in rat cortical neurons via an antioxidant mechanism that preserves mitochondrial integrity (Cherian *et al.,* 2008; Ullah *et al.,* 2012). Additionally, the treatment of TQ protected neurons from α-synuclein-induced synaptic toxicity in cultured rat primary hippocampal and human-induced pluripotent stem cell-derived neuron cells (Alhebshi *et al.,* 2014).

The present results of immunohistochemistry showed increases in the expression of iBA1, nestin and iNOS, however, synaptophysin was decreased in the brains of rats treated with VCR. Co-treatment of rats with either TQ, or EPO, or their combination restores the levels of all except synaptophysin is still lower than controls. In this aspect, Iba1 was found to be strongly expressed in activated microglia within the regenerating facial nucleus (Ohsawa *et al.,* 2000). Iba1 expression could be associated with microglial activation (Ito *et al.,* 2001) which usually occurs as an early response of the CNS against several pathological stimuli, such as; inflammation, trauma, ischemia, and degeneration. Microglia exert a cytotoxic function by releasing nitric oxide, reactive oxygen species, or inflammatory cytokines, which cause neuronal damage (González-Scarano & Baltuch, 1999; Moore & Thanos, 1996).

In multipotent CNS precursor cells, nestin represents a novel class of intermediate filament that is highly expressed (Lendahl *et al.,* 1990). The adult brain typically exhibits low levels of nestin immunoreactivity (Wei *et al.,* 2002), while nestin upregulation has been found in the lesioned brain (Bond *et al.,* 2002). Nestin is a marker for reactive astrocytes, which are important in the healing process of brain injury (Li & Chopp, 1999). Nestin is markedly induced in neuroinflammatory conditions in both astrocytes and activated microglia/ macrophages (Krishnasamy *et al.,* 2017). Post-injury nestin is highly expressed in both astrocytes and microglia, that are consistent with the hypothesis that injured cerebral tissue expressed developmental proteins, and that these proteins might aid in injury recovery (Korzhevskii *et al.,* 2008).

Synaptophysin, a calcium-binding glyco-protein located in the membranes of presynaptic vesicles of neurons, is involved in synaptogenesis, vesicular trafficking, synaptic

reorganization, and the fusion of the vesicular with the synaptic plasma membrane (Südhof, 1995). Synaptophysin is one of the most often utilized protein indicators of synaptic plasticity in the brain. The cognitive decline in Alzheimer's disease is correlated with the loss of this pre-synaptic vesicle protein in the hippocampus (Counts *et al.,* 2006; Reddy *et al.,* 2005). Synaptophysin might be associated with nerve dysfunction induced by a traumatic brain injury (Liu *et al.,* 2016). Resveratrol as an antioxidant may serve as a therapeutic strategy for traumatic brain injury via the upregulation of synaptophysin, and the inhibition of neuronal autophagy (Feng *et al.,* 2016). Activated glial cells produce more nitric oxide synthase (NOS) and RNS. (Tangpong *et al.,* 2006) and causes DNA damage in neuronal cells (Abner & McKinnon, 2004). So, natural antioxidants, resveratrol and berberine demonstrated a reversion of harmful effects of chemotherapy-induced neurotoxicity (Shaker *et al.,* 2021; Shi *et al.,* 2018). Finally, (Was *et al.,* 2022) concluded that neurotoxicity is induced through several (alone or in combination) modes of action: reduced neurogenesis and gliogenesis, direct injury of neurons, neuro-inflammation and neuroendocrine changes, hyperactivation of supportive glial cells (e.g., microglia, astrocytes, satellite glial cells), and increased oxidative stress. Consequently, supplemen-tation with antioxidants may protect against the adverse effects of chemotherapy. In conclusion, both EPO and TQ protect the cerebrum against the toxic effect of VCR by a mechanism dependent on down regulation of iNOS, IBA1, nestin, PARP and caspase 3 as mediators of oxidative stress, inflammation, and apoptosis, however, their combination gives more protection may be due to their synergetic effects.

Abbreviations

ATP:	Adenosine triphosphate	i.p:	Intraperitoneal
APS:	Ammonium persulfate	iBA1:	Ionizing calcium binding adapter molecule 1
BBB:	Blood brain barrier	mRNA:	Messenger ribonucleic Acid
B.W:	Body weight	NGF:	Nerve growth factor
CNS:	Central nervous system	NAD+:	Nicotinamide adenine dinucleotide
DNA:	Deoxyribonucleic acid	NOS:	Nitric oxide synthase
DW:	Distilled water	nDNA:	Nuclear DNA
DAB:	3,3′-diaminobenzidine	PBS:	Phosphate buffered saline
EPOR:	Erthyeropiotein receptor	PMSF:	Phenylmethylsulfonyl fluoride
EPO:	Erythropoietin	PARP:	Poly (ADP-ribose) polymerase
EDTA:	Ethylene diamine tetraacetic acid	RIPA:	Radioimmunoprecipitation assay
FDA:	Food and Drug Administration	RNS:	Reactive nitrogen species
Gran B:	Granzyme B	ROS:	Reactive oxidative stress
H&E:	Haematoxyline and Eosine	Rh-EPO:	Recombinant human erthyeropiotein
HRP:	Horse radish peroxidase	SDS:	Sodium dodecyl sulfate
iNOS:	Inducible nitric oxide synathe	SD:	Standard deviation
IAP:	Inhibitor of apoptosis protein	TQ:	Thymoquinone
IL-1:	Interleukin-1	VCR:	Vincristine

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السمية العصبية الدماغية التي يسببها الفينكريستين :التأثير التحسيني للإريثروبويتين والثيموكينون عن طريق تنظيم بروتينات iNOS و IBA1 و nestin و PARP و Caspase 3.

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الفينكريستين (VCR) دواء قوي مضاد للسرطان, على الرغم من آثاره الجانبية السامة للأعصاب. الإريثروبويتين (EPO) هو المنظم الرئيسي لتكوين الكريات الحمراء في نخاع العظام . الثيموكينون (TQ) أحد مكونات حبة البركة يحمي خلايا الدماغ من الإجهاد التأكسدي الذي يسبب الاضطرابات العصبية التنكسية مثل مرض الزهايمر ومرض باركنسون. تهدف الدراسة الحالية للبحث في سمية VCR على الدماغ والتأثير الوقائي العصبي المحتمل لـ EPO و TQ ضد سمية VCR في نموذج الجرذان. أدى الحقن داخل الغشاء البريتوني لـ VCR (١٥٠ ميكروغرام / كجم) ثلاث مرات أسبوعيًا لمدة خمسة أسابيع متتالية إلى زيادة كبيرة في التعبير الجيني لبروتينات nestin و iBA1 و iNOS ،مصحوبا بانخفاض للتعبير الجيني عن synaptophysin. علاوة على ذلك ، تسبب VCR في زيادة تعبير caspase 3 و PARP مع تغيرات نسيجية مرضية ملحوظة مثل النزف، والتنكس العصبي، وإزالة الميالين. كما اظهر علاج الفئران باستخدام EPO او TQ سواء كلا على حدة او مشتركين في مجموعة على تحسين التغييرات السابقة من خلال انخفاض في مستويات الـ nestin و iBA1 و iNOS و caspase 3 و PARP. وقد خلصت النتائج الحالية إلى أن EPO و TQ يخففان من التأثير السام العصبي لـ VCR على المخ ، وعلاوة على ذلك ، كان التأثير التآزري واضحا عندما تم الجمع بين EPO و TQ .