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AMELIORATIVE EFFECTS OF ERYTHROPOIETIN AND THYMOQUINONE ON VINCRISTINE-INDUCED BRAIN TOXICITY IN ALBINO RATS

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ABSTRACT

Vincristine (VCR) is a powerful anticancer medication, but one of its most serious adverse effects is neurotoxicity. The current experiment investigated the adverse effect of VCR on the brain and the potential neuroprotective effect of Erythropoietin (EPO) and Thymoquinone (TQ) or their combination against VCR toxicity in a rat model. The adverse effects were monitored by estimation of brain oxidative stress markers and neurotransmitters and by histopathological observation. Intraperitoneal injection of VCR (150 µg/kg) three times weekly for five consecutive weeks, significantly decreased both the level of glutathione (GSH) and the activity of acetylcholinesterase (AChE) and significantly increased the lipid peroxidation (LPO), nitric oxide (NO) and glutamate levels. Moreover, VCR caused marked histopathological changes such as neuronal degeneration, demyelination, sub-meningeal edema, hemorrhage, dilatation of brain ventricles and hyperplasia of the choroid plexus. Co-treatment of rats with EPO (80µg/kg) and their combination with TQ (10 mg/kg) improved all VCR-induced changes, however, TQ alone improved almost all changes except neurotransmitters alterations. These results suggested that the combination of EPO and TQ had an obvious neuroprotective effect against VCR neurotoxicity on oxidative stress markers, brain neurotransmitters levels and the histopathological findings in comparison with each one alone.

Keywords: Vincristine, Neurotoxicity, Erythropoietin, Thymoquinone, Neurotransmitters.

INTRODUCTION

Vincristine is a chemotherapeutic medication for the treatment of a wide human cancers, including breast, ovarian, and liver cancer as well as head and neck cancers (Moudi *et al.*, 2013). However, like many chemotherapeutic drugs, it has neurotoxic adverse effects such as neuronal and axonal degeneration, demyelination of nerve fibers and fibrosis. This is due to the VCR-induced changes in cellular microtubules resulting in changes in axonal transport and

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degeneration. The onset of VCR-induced neuropathy appears to be dose-related and early in the course of treatment. (Zhou *et al.,* 2019). The mechanism of peripheral neuropathy includes mitochondrial damage which influences the flow of Ca2+ across the mitochondrial membrane (Canta *et al.*, 2015), reduction of both the quantity and rate of Ca2+ uptake and decreasing its diffusion which alters mitochondrial function (Islam *et al.,* 2019), with increased exocytosis of neurotransmitters (Marchi *et al.*, 2018) and ROS release (Starobova and Vetter, 2017). These changes result in decreased neuronal excitability and glial function, as well as the activation of apoptosis. If these adverse effects could be avoided, VCR could be used to treat malignant tumors more successfully at higher doses and over longer periods.

Erythropoietin, a well-known hematopoietic factor in charge of red blood cells production, has a variety of activities outside the bone marrow (Lund *et al.*, 2014). When recombinant human EPO was discovered to pass the blood-brain barrier (Brines *et al.*, 2000), researchers became interested in its function in the neurological system. EPO and the EPO receptor (EPOR), which are found in a variety of tissues, exhibit pleiotropic effects on nonhemopoietic cells. EPOR was found on endothelial cells and astrocytes in the CNS, which have the ability to produce and secrete EPO (Messé *et al.,* 2013). Furthermore, EPO has been shown to be a protective or regenerative hormone that can improve cognitive performance and reverse experimental diabetic neuropathy in animal models of numerous neurological illnesses (Nekoui and Blaise, 2017).

Thymoquinone (TQ), the main component of Nigella sativa seedlings, has been found to have high anti-inflammatory, antioxidant, anticancer, immunologic and neuroprotective properties (Kooti *et al.*, 2016). TQ appears to be one of the most promising candidate medications for minimizing chemotherapy toxicity, especially given the growing interest in employing herbal medicine for the treatment of chronic illnesses.

From the foregoing literature, the present experiment was designed to evaluate the potential of EPO, TQ and their combination to protect the brain from VCR toxicity in male rats.

MATERIALS AND METHODS

1. Animals

Fifty Albino rats of 150-200g were obtained from the Laboratory Animal House, Department of Pathology, Faculty of Veterinary Medicine, Assuit University. They were given free access to food and water and kept under the standard conditions (room temperature and normal light/dark cycle) and adapted to the laboratory environment for two weeks prior to the experiment and had *ad libitum* access to commercial pellets throughout the experiment.

2. Chemicals

Vincristine sulfate (Hikma Pharmaceuticals, Egypt; 1 mg/mL vial), Human recombinant EPO (SEDICO, 6th October City, Cairo, Egypt; 10,000 IU/mL vial) and TQ (Sigma Aldrich, St Louis, MO, US). All other used chemicals were available with high purity.

3. Experimental design

Rats were divided into 5 equal groups: Group I (control group), received ringer's solution and DMSO as a vehicle for 5 consecutive weeks. The other 4 groups were treated with an intraperitoneal injection of 150µg/kg VCR three times weekly for 5 consecutive weeks (Ja'afer *et al.*, 2006). Group II (VCR group) was left as positive control without any treatment. Group III (EPO group) was treated concomitantly with an intraperitoneal injection of 80µg/kg of EPO (Kassem *et al.*, 2011). Group IV (TQ group) was orally treated concomitantly with 10mg/kg of TQ

(Mehri *et al.,* 2014). Group V (EPO+ TQ group) was treated concomitantly with EPO and TQ at the same route of administration and doses as in groups III and IV.

At the end of the experiment, rats in all groups were sacrificed for collection of the brain after infusion with 10 % phosphate-buffered formalin. Part of the brain tissue was immersed in 10 % phosphate-buffered formalin for histopathological examination and the other parts were preserved at -80 ℃ for biochemical estimation of oxidative stress markers (LPO, NO and GSH) and brain neurotransmitters (AChE and glutamate).

4. Biochemical estimation

Brain tissues were rinsed in PBS to remove excess blood thoroughly and weighed before homogenization. Brain tissues were minced and homogenized in PBS (0.1 M, pH7.4) by using a homogenizer (IKA Yellow line DI 18 Disperser, Germany), then centrifuged at 3000g for 20 minutes and the supernatant was stored at -20°C for estimation of oxidative stress markers (LPO, NO and GSH) and brain neurotransmitters (glutamate and AChE) in the Physiology Lab. of Zoology Department, Faculty of Science, Assiut University.

5. Histopathological examination

After fixation, specimens were washed with running tap water, dehydrated in ascending ethyl alcohol grades, cleared with xylene, infused and embedded in paraffin wax. Sections of 4µm were cut s, mounted on slides, dried overnight at 37 °C and then processed for hematoxylin and eosin staining (Suvarna *et al.,* 2013) and Luxol Fast Blue (LFB) stain (Goto 1987). The stained sections were examined under the light microscope (Olympus CX31, Japan) and photographed using a digital camera (Olympus, Camedia C-5060, Japan) in the photographing Lab. of Pathology & Clinical Pathology Department, Faculty of Veterinary Medicine, Assiut University.

6. Statistical analysis

Statistical analysis was done using analysis of variance (ANOVA) followed by Duncan's Multiple Comparison Test as post-Test by using IBM SPSS statistics, version 20. All Data were presented as mean ± SEM. and the level of significance was set at $p<0.05$.

RESULTS

1. Biochemical estimation

1.1.Oxidative stress markers

The levels of LPO and NO exhibited a significant elevation, however, GSH level was significantly decreased in the VCR-treated group ($p < 0.05$) when compared with the control one. In all co-treated groups with EPO, TQ, and their combination all the previous changes in oxidative stress markers were non significantly changed in comparison with the control group (Table 1).

Table 1: Oxidative stress markers in brain tissue of the five experimental groups.

	Control	VCR	EPO	TQ	VCR&EPO &TQ
LPO (nmoles/mg proteins)	5.8 ± 0.37^{b}	8.24 ± 0.5^{a}	6.18 ± 0.19^{b}	6.07 ± 0.38^{b}	5.15 ± 0.25^{b}
NO (nmoles/mg proteins)	32.48 ± 0.7^{b}	49.20 ± 1.81^{a}	33.30 ± 1.15^{b}	34.52 ± 0.9^{b}	33.73 ± 1.02^{b}
GSH (nmoles/mg proteins)	2.56 ± 0.13^{a}	1.64 ± 0.18^{b}	2.63 ± 0.13^{a}	2.13 ± 0.08^{a}	2.26 ± 0.5^{a}

Data were expressed as the mean ± S.E, where n=6. Means within the same row with different superscripts were significantly different at $P< 0.05$.

1.2. Brain neurotransmitters

The level of glutamate showed a significant elevation; however, AChE activity was significantly decreased in the VCR-treated group ($p < 0.05$) when compared with the control one. In the EPO-treated and EPO&TQ-treated groups, the glutamate and AChE levels were improved more than in the VCR group but decreased when compared with the control group. However, in the TQ-treated group, the glutamate level was increased, and the AChE level was decreased when compared with the VCR group (Table 2).

Table 2: Neurotransmitters, acetylcholinesterase activity and glutamate level in the five experimental groups.

	Control	VCR	EPO	TQ	VCR&EPO &TQ
Glutamate µg/mg proteins	3.74±0.09 ^{c}	7.43±0.31^{b}	4.08±0.24^{c}	9.92±0.27^{a}	3.80±0.11^{c}
AChE µmoles/mg proteins	7.48±0.3 ^{a}	4.92±0.13 ^{c}	6.08±0.3 ^{b}	4.56±0.03 ^{c}	6.17±0.29 ^{b}

Data were expressed as the mean ± S.E, where n=6. Means within the same row with different superscripts were significantly different at $P< 0.05$

2. Histopathological findings

2.1.Control –ve group:

Light microscopy of the brain tissue showed a normal morphological appearance without any histological changes (Fig. 1A).

2.2.VCR group:

Light microscopic findings of the brain tissue revealed that I.P. injection of **VCR** induced several histopathological changes in the cerebrum and cerebellum. The cerebral changes were in the form of severe neuronal degeneration, demyelination of nerve fibers, sub-meningeal edema and hemorrhage, dilatation of brain ventricles, congestion of blood vessels and perivascular edema. Hyperplasia of the choroid plexus also was seen in the cerebrum in some cases. The cerebellum changes were in form of degeneration of Purkinje cells, demyelination, congestion of blood vessels and perivascular sub-meningeal hemorrhage (Fig. 1B, C, D, E, F and Fig. 2).

2.3. VCR&EPO group:

Light microscopy of the brain tissue of rats belonging to this group revealed a marked decrease in the histopathological changes which were observed in the **VCR** group. These changes were manifested by the presence of mild neuronal degeneration, demyelination, dilatation of brain ventricles, congestion of blood vessels and perivascular edema. Mild hyperplasia of the choroid plexus was seen in this group than in the **VCR** group. Sub-meningeal edema and hemorrhage were seen with the same level shown in the **VCR** group. Also, there was a moderate loss of Purkinje cells, degeneration of Purkinje cells and demyelination in the cerebellum. However, the normal morphological appearance of most neurons was observed (Fig. 3A, B and Fig. 4A, B).

2.4. VCR&TQ group:

Light microscopy of the brain tissue of this group nearly showed the same appearance as **VCR** brains. Congestion of blood vessels and perivascular edema in the cerebrums were the same as in the **VCR** group. Severe hyperplasia of the choroid plexus and dilatation of ventricles were more seen in

this group than in the **VCR** group. However, there was moderate neuronal degeneration. Also, there were mild sub-meningeal edema and hemorrhage. The cerebellum changes were in form of degeneration or even marked loss of Purkinje cells, demyelination, congestion of blood vessels and perivascular sub-meningeal hemorrhage (Fig. 3C, D and Fig. 4C, D).

2.5. VCR, EPO&TQ group:

Light microscopy of the brain tissue of rats belonging to this group revealed a marked decrease in the histopathological changes observed in the **VCR** group. These changes were manifested by the presence of mild neuronal degeneration, sub-meningeal edema, congestion of blood vessels and perivascular edema. Sub-meningeal hemorrhage was seen within the same level shown in the **VCR** group. Mild hyperplasia of the choroid plexus was more seen in this group than **VCR** group. Also, degeneration or even mild loss of Purkinje cells and demyelination in the cerebellum had been observed. However, normal morphological appearance of the neurons, brain ventricles and cerebellum were observed (Fig. 3E, F and Fig. 4E, F).

Table 3: Scoring of histopathological findings in brain tissue of the different groups.

Pathological findings	Control	VCR	VCR+ EPO	VCR+ TQ	VCR+ EPO+ TQ
Sub-meningeal edema	-ve	+++	+++	+	+
Sub-meningeal hemorrhage	-ve	+++	+++	++	+++
Dilatation of brain ventricles	-ve	+++	++	+++	-ve
Congestion of blood vessels	-ve	+++	++	+++	++
Perivascular edema	-ve	++	+	++	+
Neuronal degeneration	-ve	+++	+	++	+
Degeneration and loss of some Purkinje cells	-ve	+++	++	+++	+
Hyperplasia of choroid plexus	-ve	+++	+	+++	+

Intensity scores: → -ve = Not found, + = Mild, ++ = Moderate, +++ = Severe.

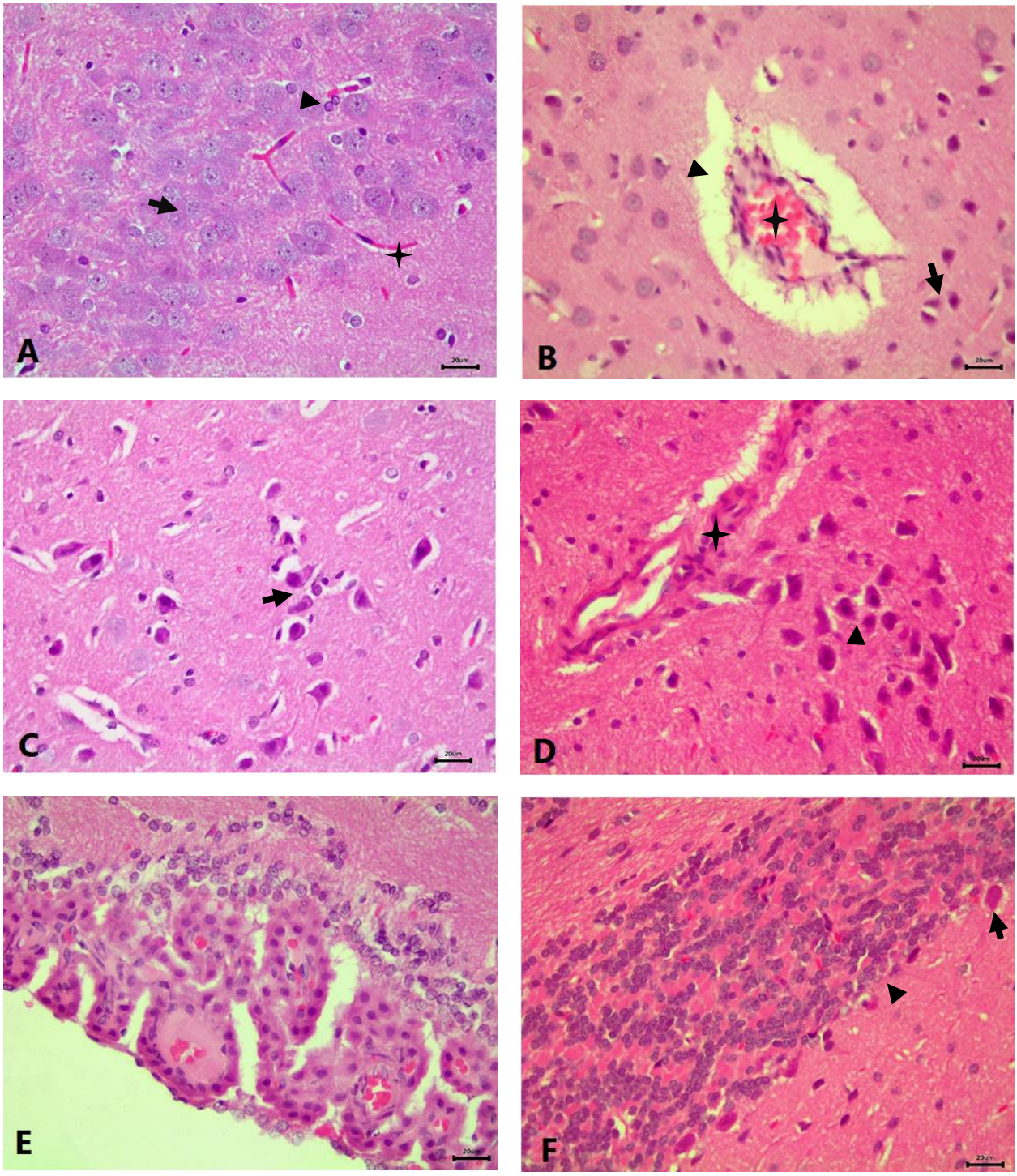

Fig. 1: (A) Brain of the control group showing a normal morphological appearance of neurons (arrow), glial cells (arrowhead) and blood vessels (star). (B) The brain of the VCR group showing neuronal degeneration (arrow), congestion of blood vessels (star) and perivascular edema (arrowhead). (C) severe neuronal degeneration with aggregations of neuroglia around the degenerated neurons (satellitosis) (arrow). (D) severe neuronal degeneration (arrowhead) with vasculitis (star). (E) severe hyperplasia associated with papillation of choroid plexus. (F) nuclear pyknosis of Purkinje cells (arrow) with loss of some Purkinje cells (arrowhead) (H&E, bar= 20 um).

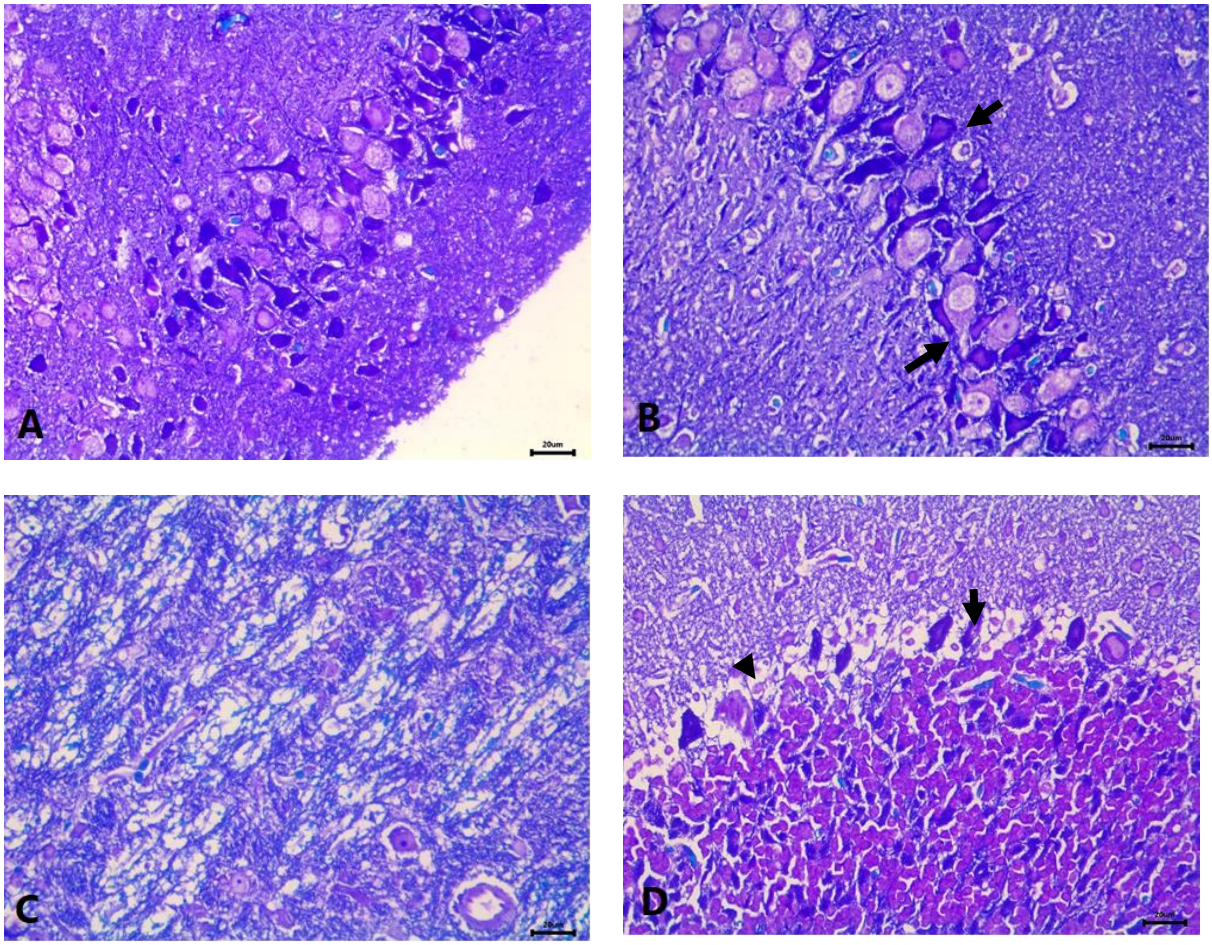

Fig. 2: Brain of VCR group showing (A) severe nuclear pyknosis and chromatolysis of neurons with the piercing of Nissl granules, (B) Hippocampus, some neurons with nuclear pyknosis (arrow), (C) Demyelination in white matter and (D) Cerebellum, shrinkage and nuclear pyknosis of Purkinje cells (arrow) with marked loss of some Purkinje cells (arrowhead) (LFB, bar=20um).

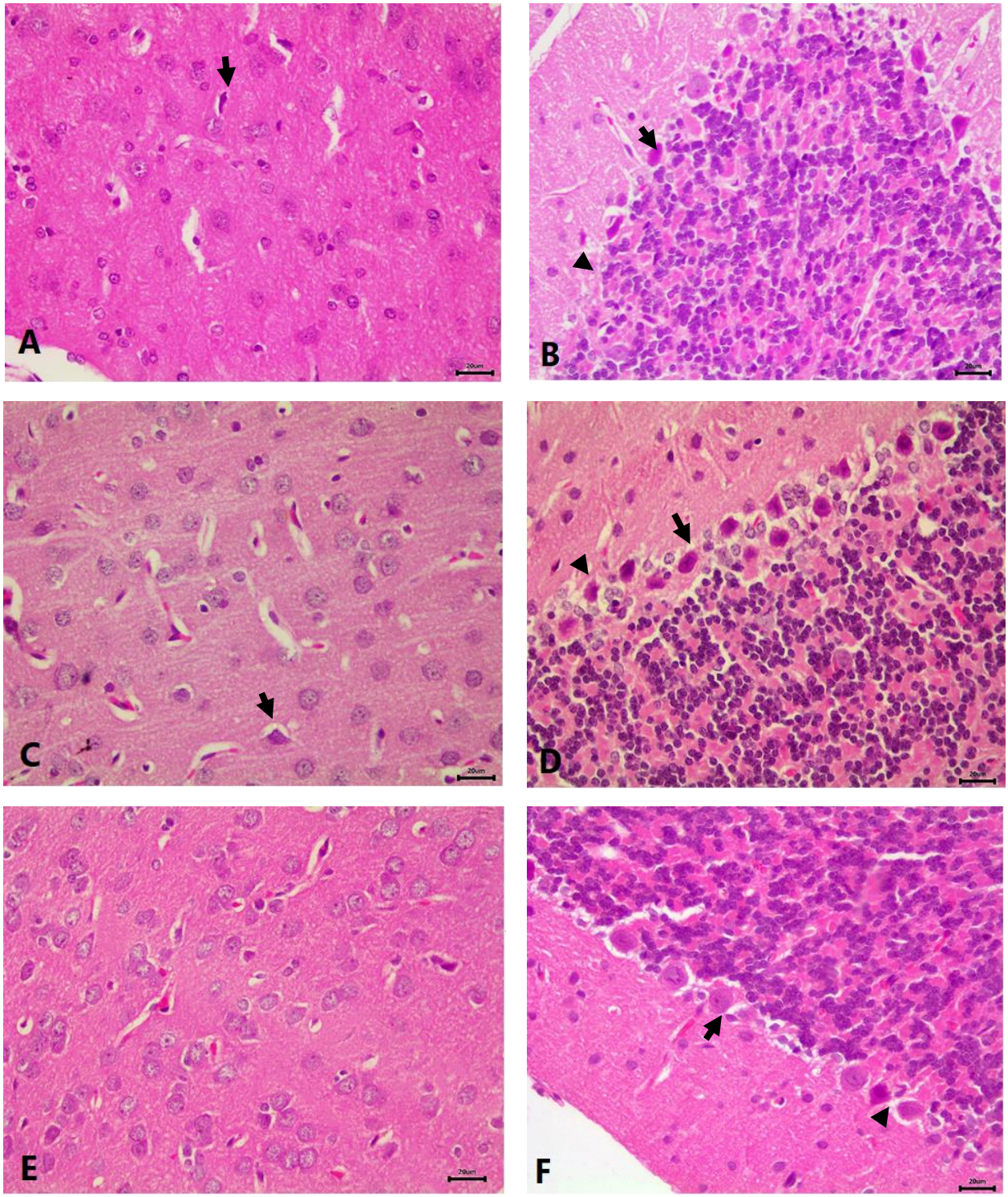

Fig. 3: Brain of EPO group showing: (A) normal neurons with mild neuronal degeneration (arrow) and (B) moderate degeneration of Purkinje cells (arrow) with loss of some Purkinje cells (arrowhead). The brain of the TQ group showing: (C) normal neurons with mild neuronal degeneration (arrow) and (D) nuclear pyknosis of Purkinje cells (arrow) with shrinkage of some Purkinje cells (arrowhead). The brain of the EPO&TQ group showing: (E) normal neurons and (F) normal Purkinje cells (arrow) with shrinkage and chromatolysis of some Purkinje cells (arrowhead) (H&E, bar= 20 um).

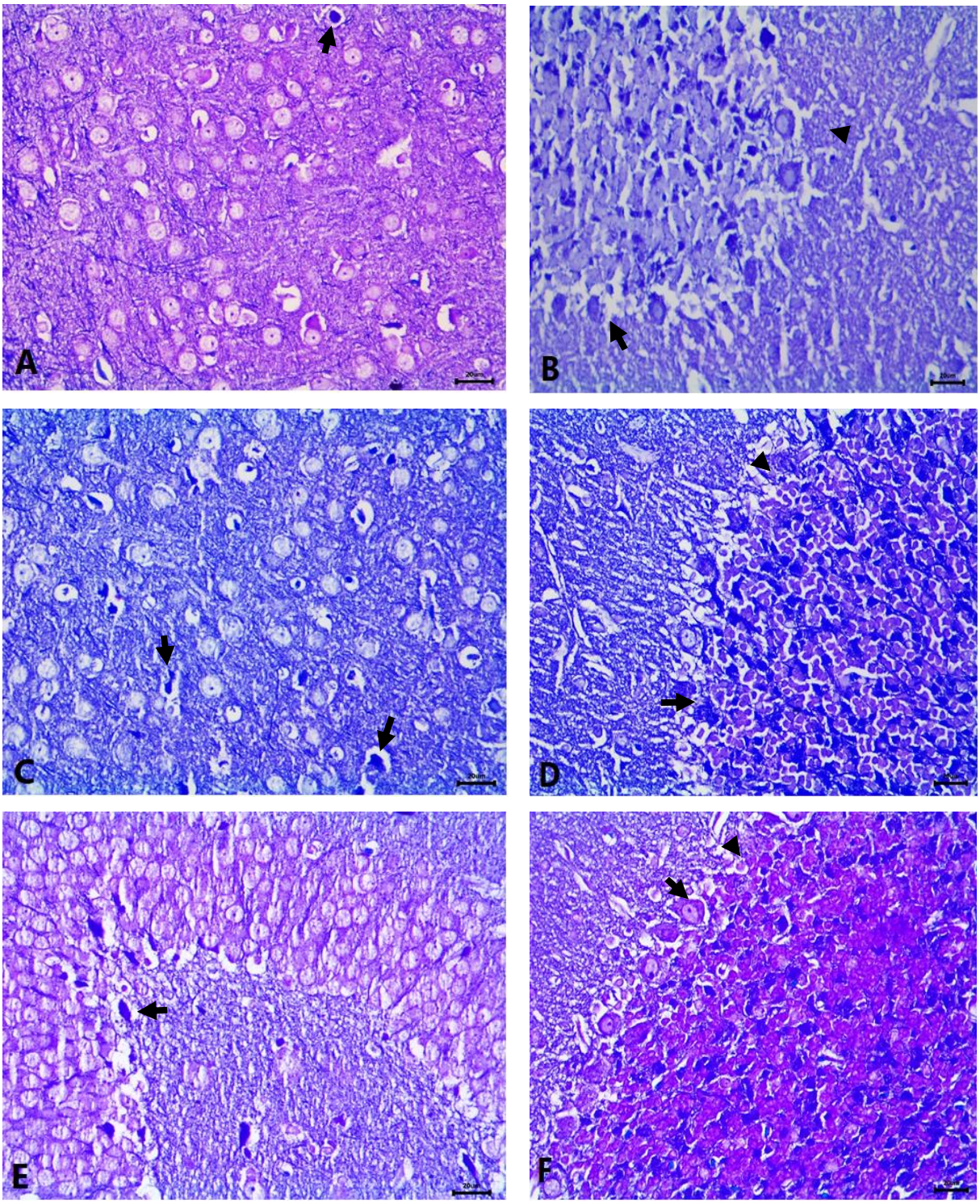

Fig. 4: Brain of EPO group showing: (A) normal neurons with mild neuronal degeneration (arrow) and (B) normal Purkinje cells (arrowhead) with mild nuclear pyknosis of some Purkinje cells (arrow). The brain of the TQ group showing: (C) normal neurons with moderate neuronal degeneration (arrow) and (D) nuclear pyknosis and chromatolysis of Purkinje cells (arrow) with marked loss of some Purkinje cells (arrowhead). The brain of the EPO&TQ group showing: (E) normal neurons with mild neuronal degeneration (arrow) and (F) normal Purkinje cells (arrow) with loss of some Purkinje cells (arrowhead) LFB, bar=20um).

DISCUSSION

VCR is one of the most commonly used potent antineoplastic agents for the treatment of a wide range of cancers (Moudi *et al.,* 2013). Despite its excellent anticancer activity, its clinical use is often limited by its undesirable severe toxic effects that interfere with its therapeutical efficacy (Zhou *et al.,* 2019). various gents have been used in clinical and experimental studies to protect against VCR neurotoxicity, however, none of them have been proven to be effective as a complete chemo-preventive barrier in patients (Triarico *et al.,* 2021). The present experiment was designed to study the neurotoxic effects of VCR on the rat model which is the most suitable animal model for evaluation of brain lesions caused by VCR (Gadgil *et al.,* 2019) and to study the efficacy of EPO, TQ and both to protect against VCR toxicity.

Mitochondria are engaged in calcium signaling, cell death, membrane regulatory potential, and cellular metabolism. As a natural consequence of oxygen metabolism, mitochondria in healthy tissues produce small amounts of ROS. These radicals play critical roles in cell signaling. Most chemotherapeutic agents cause mito-chondrial damage in neuronal and non-neuronal cells, resulting in increased ROS generation and thus inducing oxidative stress (McDonald and Windebank, 2002). The pathological increase in ROS generation in order causes damage to intracellular biomolecules like enzymes, proteins and lipid molecules leading to peripheral nerves demyelination (Zheng *et al.,* 2011). Moreover, ROS can activate apoptotic pathways, increase pro-inflammatory mediators production and cause oxidative stress pathology (Areti *et al.,* 2014).

In the present study, administration of VCR caused a significant increase in brain LPO and NO, however, GSH was significantly decreased in comparison with control. Similarly, in comparison with control mice, VCR-treated mice exhibited a significant elevation in LPO and NO levels and a significant decrease in GSH (Anand Babu *et al.,* 2015). Also, cisplatin treatment resulted in massive organ damage, as evidenced by a significant elevation in tissue MDA level compared with the control group (Ilbey *et al.,* 2009). MDA is a byproduct of LPO and a marker of ROS production which causes more advanced cellular injury (Ilbey *et al.,* 2009). It is known that VCR alters the mitochondrial function with consequent release of ROS (Starobova and Vetter, 2017) resulting in an increase in LPO and NO and a decrease in GSH. Lipid hydroperoxides are intermediates of peroxidative reactions that have a longer half-life than any free radical precursors. As a result, LOOH toxicity may manifest far outside the site of LOOH origin (Girotti1, 1998). NO is involved in the preservation and regulation of normal neuronal functions, including those associated with toxic conditions. Chronic treatment of rats with VCR produces alterations in the expressions of eNOs and iNOS (Herradón *et al.,* 2021). Moreover, nNOS up-regulates NO, which activates hypoxia-inducible factor-1, a key regulator of EPO in Schwann cells (Hoke, 2006) and protects the neuron and axon from injury. This neuroprotective pathway can conquer toxic injury to some extent, but it is easily supplemented by exogenous EPO, as demonstrated in paclitaxel-induced peripheral neuropathy (Melli *et al.,* 2006). GSH is essential for cell differentiation, propagation, and cell death. GSH homeostasis disruptions play a role in the etiology and advancement of many diseases. So, a decrease in the GSH results in higher vulnerability to oxidative stress, which has been linked to cancer progression (Traverso *et al.,* 2013).

Co-administration with EPO, TQ or their combination almost normalized the alteration in LPO, NO and GSH. In

consistence with these results, Anand Babu *et al.* (2015) found that curcumin caused a significant decrease in LPO and NO and an increase in endogenous antioxidant enzymes. Also, quercetin is protective against VCR-induced peripheral neurotoxicity due to relieving oxidative stress and neuronal cell damage (Yardim *et al.*, 2020). Increased GSH levels boost antioxidant potential and resistance to oxidative stress, as seen in many cancer cells (Traverso *et al.*, 2013). So, Colla *et al*. (2016) recommended that monitoring GSH levels is an effective strategy for detecting the occurrence of drug resistance and controlling the patient's response to chemotherapy.

Acetylcholine is a neurotransmitter, found in both the central and peripheral nervous system that transmits signals from nerve endings to terminal glands and muscles. AChE is an enzyme that breaks down acetylcholine into choline and acetate. AChE is a target for many drugs and toxins (Krall *et al.*, 1999). Direct axonal damage, mitochondrial dysregulation, and alteration in the gene expression of pain mediators, including neurotransmitters, ion channels, and growth factors, are among the suggested mechanisms of chemotherapy-induced peripheral neuropathy (Fehrenbacher, 2015). Furthermore, in patients undergoing chemotherapy, there is a link between both the severity of chemotherapy neuropathy and decreasing levels of nerve growth factor (De Santis *et al.*, 2000). Excitotoxic glutamate release has been linked to neuronal damage and death in a variety of neurological disorders. N-Acetyl-aspartyl-glutamate is a common neuropeptide found throughout the central and peripheral nervous systems that is biologically hydrolyzed into N-Acetyl-aspartyl and glutamate by the enzyme glutamate carboxypeptidase. Thus, inhibiting glutamate carboxypeptidase significantly improved chemotherapy-induced nerve conduction velocity deficits (Carozzi *et al.*, 2009). In the present study, co-administration of rats with EPO, TQ, or their combination almost normalized the level of glutamate and the activity of AChE. Similarly, caffeine has the potential to act as an inhibitor of AChE in the body (Pohanka & Dobes, 2013). Hence, AChE inhibitors are effective for the treatment of mild to moderate Alzheimer's disease (Geerts & Grossberg, 2006).

Morphological observation revealed that VCR induced several histopathological changes in the cerebrum and cerebellum. The cerebrum changes were in form of severe neuronal degeneration, sub-meningeal edema and hemorrhage, dilatation of brain ventricles, congestion of blood vessels and perivascular edema. Hyperplasia of the choroid plexus also was seen in some cerebrums. The cerebellum changes were degeneration of Purkinje cells and demyelination. These findings were similar to the findings of Starobova and Vetter (2017) and Lee and Hur (2020). Histopathological findings in brain tissues were confirmed by the increase of the neurotransmitters glutamate and the activity of AChE and the changes in oxidative stress markers (LPO, NO & GSH) when compared with the control group. Similari results were reported by many studies (Gautam and Ramanathan 2019; Meléndez *et al.*, 2020; Herradón *et al.*, 2021).

Co-administration of EPO with VCR and the combination of EPO with TQ caused marked improvement of the histo-pathological changes which were observed in the brain of the VCR group with the presence of mild neuronal degeneration, dilatation of brain ventricles, perivascular oedema and hyperplasia of choroid plexus. Moreover, a moderate degeneration of Purkinje cells and demyelination in the cerebellum were seen. Similarly, EPO improves regeneration after injury by ischemia and reperfusion injury and protects against neuropathic pain (Nekoui and Blaise, 2017). On the contrary, Fan *et al.* (2013) reported that EPO showed no

effect on brain tissue loss and white matter damage. These histopathological improvements were confirmed by the restoration of neurotransmitter levels of glutamate and AChE and the oxidative markers (LPO, NO & GSH) were consistent with many previous studies (Mohamed *et al.,* 2018; Liu *et al.,* 2019; Samson *et al.,* 2020).

Co-administration of TQ with VCR caused slight improvements in the histopathological changes observed in the VCR group. These changes were manifested by moderate neuronal and axonal degeneration, mild sub-meningeal edema and hemorrhage. Similarly, Radad *et al.* (2014) found that TQ markedly decreased the incidence of neuronal degeneration, cerebellar neurodegeneration and demyelination. Also, Abulfadl *et al.* (2018) stated that TQ effectively reduced neuronal cell death, which is a major cause of the huge neuronal loss, by increasing the release of the anti-apoptotic factor Bcl-2. The slight improvement in the brain histopathological changes was consistent with the improvement in the oxidative stress makers levels in the brain in comparison with the VCR group which is in agreement with many studies (Atta *et al.,* 2018; Safhi *et al.,* 2019; Fanoudi *et al.,* 2019). However, in the present study, glutamate and AChE levels were the same as VCR which in contrast with Sanati *et al.* (2018); Aboubakr *et al.* (2021) who stated that TQ decreased the elevation of brain glutamate and improved AChE activity.

In conclusion, accumulative administration of VCR to male rats caused marked histopathological changes in the brain mediated by a significant change in oxidative stress makers and neurotransmitters. Co-administration with either EPO or the combination of EPO with TQ markedly improved the histopathological in the brain due to the amelioration of oxidative stress makers and neurotransmitters, however, co-administration with TQ slightly improved the histopathological in the brain due to the amelioration of oxidative stress makers, however not restored the neurotransmitters.

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التأثيرات المحسنه للإريثروبويتين والثيموكينون على السمية العصبية المحدثة بالفينكريستين فى الجرذان البيضاء

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الفينكريستين، دواء مضاد للسرطان، وتعتبر السمية العصبية واحدة من أخطر آثاره الضارة. إستقصت التجربة الحالية التأثير الضار للفينكريستين على المخ والتأثير الوقائي العصبي المحتمل للإريثروبويتين والثيموكينون أو توليفة منهما ضد سمية الفينكريستين في الجرذان. تم تقييم الآثار الضارة عن طريق تقدير علامات الإجهاد التأكسدي لنسيج المخ والناقلات العصبية ومن خلال الفحص الباثولوجي. بعد الحقن في التجويف البريتوني للفينكريستين (150ميكروغرام / كجم) ثلاث مرات أسبوعيًا لمدة خمسة أسابيع متتالية، انخفض معنويا مستوى الجلوتاثيون (GSH) ونشاط أستيل كولينستريز (AChE) وزاد معنويا كلا من أكسدة الدهون(LPO) ، أكسيد النيتريك (NO) والناقل العصبي الجلوتامات. علاوة على ذلك، تسبب الفينكريستين في تغيرات نسيجية مرضية مثل احتقان الأوعية الدموية والوذمة تحت السحائية، والضمور العصبي، وإزالة الميالين، وتوسع البطينات الدماغية، وتضخم الضفيرة المشيمية (choroid plexus). بينما أدت المعالجة المشتركة للجرذان باستخدام الإريثروبويتين (80 ميكروغرام / كجم) أو توليفها مع الثيموكينون (10مجم / كجم) إلى تحسين جميع التغييرات التي احدثها الفينكريستين، ومع ذلك، حسنت الثيموكينون وحدها جميع التغييرات تقريبًا باستثناء تغييرات الناقلات العصبية. تشير هذه النتائج إلى أن الجمع بين الإريثروبويتين والثيموكينون كان له تأثير وقائي واضح ضد السمية العصبية للفينكريستين.