Terminalia muelleri extract supplementation alleviates doxorubicin-induced neurotoxicity in rats: involvement of oxidative stress and neuroinflammation, apoptosis, extracellular signal-regulated kinase, and mammalian target of rapamycin

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Background
Doxorubicin (DOX) is widely used to treat many human cancers, but significant brain damage limits its clinical application.
Objectives
To investigate the neuroprotective activity of *Terminalia muelleri* extract (TME) against DOX-induced neurotoxicity in rats.
Materials and methods
The first group served as a normal control; the second group served as a positive control which was treated with DOX (2.5 mg/kg; dissolved in saline; intraperitoneal three times/week for 2 weeks,); the third group was treated with TME at a dose of 100 mg/kg; the fourth group was pretreated with TME for 2 weeks and then coadministrated with DOX for other 2 weeks; the fifth and sixth groups were treated with DOX for 2 weeks and then posttreated with two doses of TME (100, 200 mg/kg), respectively, for another 2 weeks. The experiment lasted for 4 weeks; brain tissue samples were harvested for the measurement of toxicity such as oxidative stress, inflammation, apoptosis, neurodegeneration, and histopathological examinations.
Results and conclusion
DOX-treated animals showed a reduction in glutathione and superoxide dismutase along with a raise in malondialdehyde, nitric oxide, and myeloperoxidase. Also, it caused an increase in caspase-3, indicating an increased propensity for cell death, acetylcholinesterase, extracellular signal-regulated kinase, mammalian target of rapamycin with concomitant decrease in brain-derived neurotrophic factor. However, administration of TME significantly improved oxidative stress alterations, brain-derived neurotrophic factor, and apoptosis. Histological assessments of brain tissues supported the obtained biochemical finding. In conclusion, our findings disclose a potent protective role of TME by activating antioxidant, anti-inflammatory, anti-apoptotic, and neurogenesis effects, which may contribute to the safe use of DOX in cancer treatment.

Keywords:
doxorubicin, brain damage, oxidative stress, apoptosis, *Terminalia muelleri* extract

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Introduction

Chemotherapy is one of the best remedial strategies in the treatment of tumors. The efficacy of chemotherapeutic drugs has produced an enormous increase in the number of cancer survivors [1], regarding that, chemotherapeutic drugs do not only treat malignant cells, but also cause various adverse side effects on healthy body cells during the treatment [2].

Doxorubicin (DOX, adriamycin), one of the most potent antineoplastic agent of the anthracycline group, is widely used in the treatment of various human malignancies as solid tumors and leukemia [3,4]. The antitumor effect of DOX has been reported to be mediated by blocking replication of DNA, the formation of free radicals, and lipid peroxidation in cancer cells [5–7]. However, its clinical application is restricted due to the considerable cytotoxicity in nontarget tissues such as the liver, kidneys, heart, and brain [8–11]. Production of hydroxyl radicals and superoxide radicals along with hydrogen peroxide after the administration of DOX causes alterations in the oxidant–antioxidant system [12]. Therefore, it has been reported that long-term administration of DOX resulted in some adverse effects as heart arrhythmias, cardiotoxicity, kidney injury, and neurotoxicity [13–15].

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Although DOX poorly transport across the blood–brain barrier, after systemic administration it penetrates to circumventricular organs of the brain and stimulates degenerative injury in these areas [16,17]. Previous studies have demonstrated that DOX decreased hippocampal neurogenesis, enhanced inflammations, caused neural apoptosis, and induced depression-like behaviors in rats [18–20].

DOX by itself weakly stimulates cellular defense against oxidative stress [21]; this increases the need for the application of efficacious antioxidative neuroprotectors. Therefore, several studies were carried out for the screening of the antioxidants extracted from natural sources aiming to reducing oxidative damage by DOX [22,23]. In this regard, the majority of these antioxidants were used to amend the deleterious effect of DOX on healthy cells without reducing drug dosage or affecting its anticancer efficacies [24].

Medicinal plants have attracted much attention for centuries to date as an alternative therapy useful for treating various diseases; so there has been a growing interest in searching for new antioxidants from botanical sources. Many Egyptian plants were used in traditional medicine long time ago, which encouraged many researchers to direct their attention in carrying out many studies on these plants [25]. One of these plants is *Terminalia muelleri* Benth, (family: Combretaceae). It is a flowering plant and is distributed in India, Indonesia, and North America along with a commonly known Australian almond [26]. *T. muelleri* is widely used in traditional medicine because of its high content of different secondary metabolites such as phenolic acids, flavonoids, tannins, gallic and ellagic acids, and other compounds [26,27]. Because of these phytoconstituents, it has wide pharmacological activities as antioxidants, antidiabetic, anti-inflammatory, and hepatoprotective [27–30].

On the basis of above findings, the present study was planned to throw light on the possible neuroprotective effect of *Terminalia muelleri* extract (TME) on DOX-induced neurotoxicity.

Materials and methods

Animals

The present experimental study was carried out on white albino rats (*Rattus norvegicus*). The standard guidelines of National Organization for Drug Control and Research (NODCAR) were used in handling animals. The animals were selected from a pure strain, so genetic influence was kept at a constant and uniform level. Animals had free access to food and water *ad libitum*. They were maintained at 21–24°C and 40–60% relative humidity with 12-h light–dark cycle. All animal procedures were performed in accordance to the Institutional Ethics Committee and in accordance with the recommendations for the proper care and use of laboratory animals. Unnecessary disturbance of animals were avoided. Animals were treated gently; squeezing, pressure, and tough maneuver were avoided. All procedures were carried out according to the Research Ethics Committee for experimental studies at the National Organization for Drug Control and Research NODCAR/I/1/2020 on February 19, 2020.

Plant material

The leaves of *T. muelleri Benth.* were collected from Giza Zoo. The plant was botanically authenticated by the taxonomy specialist at the herbarium of El-Orman botanical garden, Giza, Egypt, and it was deposited at the Division of Biochemistry, NODCAR.

Extraction

The shade-dried powdered leaves were extracted three times with 80% aqueous ethanol. The extract was separated by filtration and the pooled filtrates were concentrated under reduced pressure in a rotary vacuum evaporator, yielding a dried residue Then, the solid residue was stored at 4°C for subsequent experiments. The dried matter was suspended in saline for using in the experimental studies [27].

Drugs

DOX vial (50 mg/25 ml) was purchased from Ebewe Pharma, Austria Company (Unterach, Austria) and used in a dose (2.5 mg/kg; intraperitoneal; three times weekly for 2 weeks) [31].

Experimental design

After 2 weeks of acclimatization to the laboratory environment, 36 albino rats of nearly similar weights of between 180 and 200 g were randomly selected and divided into control and treated groups. The study pattern was designed in the following manner:

Group 1: served as normal control.
Group 2: rats were injected with DOX (2.5 mg/kg; dissolved in saline; intraperitoneal) three times/week for 2 weeks [31], and these animals served as a positive control group.
Group 3: rats treated with a daily oral dose of TME (100 mg/kg body weight) for 4 weeks [27].

Group 4: rats treated with a daily oral dose of TME (100 mg/kg) for 2 weeks before DOX injection and then coadministered with DOX (2.5 mg/kg; dissolved in saline; intraperitoneal) three times/week for 2 weeks for another 2 weeks.
Group 5: rats were injected with DOX (2.5 mg/kg; intraperitoneal) three times/week for 2 weeks and then rats were treated with a daily oral dose of TME (100 mg/kg) for another 2 weeks.
Group 6: rats were injected with DOX (2.5 mg/kg; intraperitoneal) three times/week for 2 weeks and then rats were treated with a daily oral dose of TME (200 mg/kg) for another 2 weeks.

At the end of the experiment, 24 h after last manipulation, the animals were decapitated, and then brains were isolated. The dissected brains were harvested and rinsed with ice-cold isotonic saline. Brains were divided into two portions; one was kept in 10% formalin for histopathological examinations while the other part was kept in −80°C for estimating the other biochemical parameters. The cerebral cortex (which included the hippocampus) will be dissected; each of them will be weighed and homogenized in ice-cold PBS to prepare 10% homogenate that will be used for the assessment of oxidative stress biomarkers [glutathione (GSH), superoxide dismutase activity (SOD), malondialdehyde (MDA)], inflammatory marker [myeloperoxidase (MPO), nitric oxide (NO)], brain-derived neurotrophic factor (BDNF) apoptotic marker (caspase-3), extracellular signal-regulated kinase (ERK), and mammalian target of rapamycin (m-TOR).

Biochemical analysis

(1) Estimation of cerebrum reduced GSH contents:
The content of GSH was measured as nonprotein thiols based on the protocol developed by Beutler *et al.* [32].
(2) Estimation of cerebrum SOD:
The activity of SOD was determined in the homogenate using a Biodiagnostic Kit (Cairo, Egypt) according to the method described by Kinouchi *et al.*[33].
(3) Estimation of cerebrum MDA contents:
The determination of MDA was carried out according to the method of Buege and Aust [34].
(4) Estimation of cerebrum inflammatory activity of MPO:
MPO activity was done using the kinetic colorimetric method described by Bradley *et al.* [35].
(5) Estimation of inflammatory response of NO in the cerebrum.
Cerebrum NO colorimetric assay was performed using a Biodiagnostic reagent test kit according to the method of Montgomery and Dymock [36].
(6) Determination of cerebrum BDNF, ERK, and m-TOR:
Enzyme-linked immunosorbent assay was used to determine cerebrum BDNF, ERK, and m-TOR by using a reagent test kit (Bioassay Technology Laboratory, Shanghai, China), according to the manufacturer's instructions.
(7) Determination of cerebrum apoptotic factor (caspase-3) content:
Enzyme-linked immunosorbent assay was used to determine cerebrum caspase-3 by using a test reagent kit (Bioassay Technology Laboratory) according to the manufacturer's instructions.
(8) Determination of cerebrum acetylcholinesterase (AChE) activity:
The activity of AChE was determined using DTNB phosphate reagent after 10 min incubation of the cerebrum homogenate with acetyl thiocholine iodide [37].

Histopathological examination

Autopsy samples were taken from the brain of rats in different groups and fixed in 10% formalin saline for 24 h. Washing was done in tap water and then serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56° in hot air ovens for 24 h. Paraffin beeswax tissue blocks were prepared for sectioning at 4 μm thickness by a sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained using hematoxylin and eosin stain for examination through the light electric microscope [38].

Statistical analysis

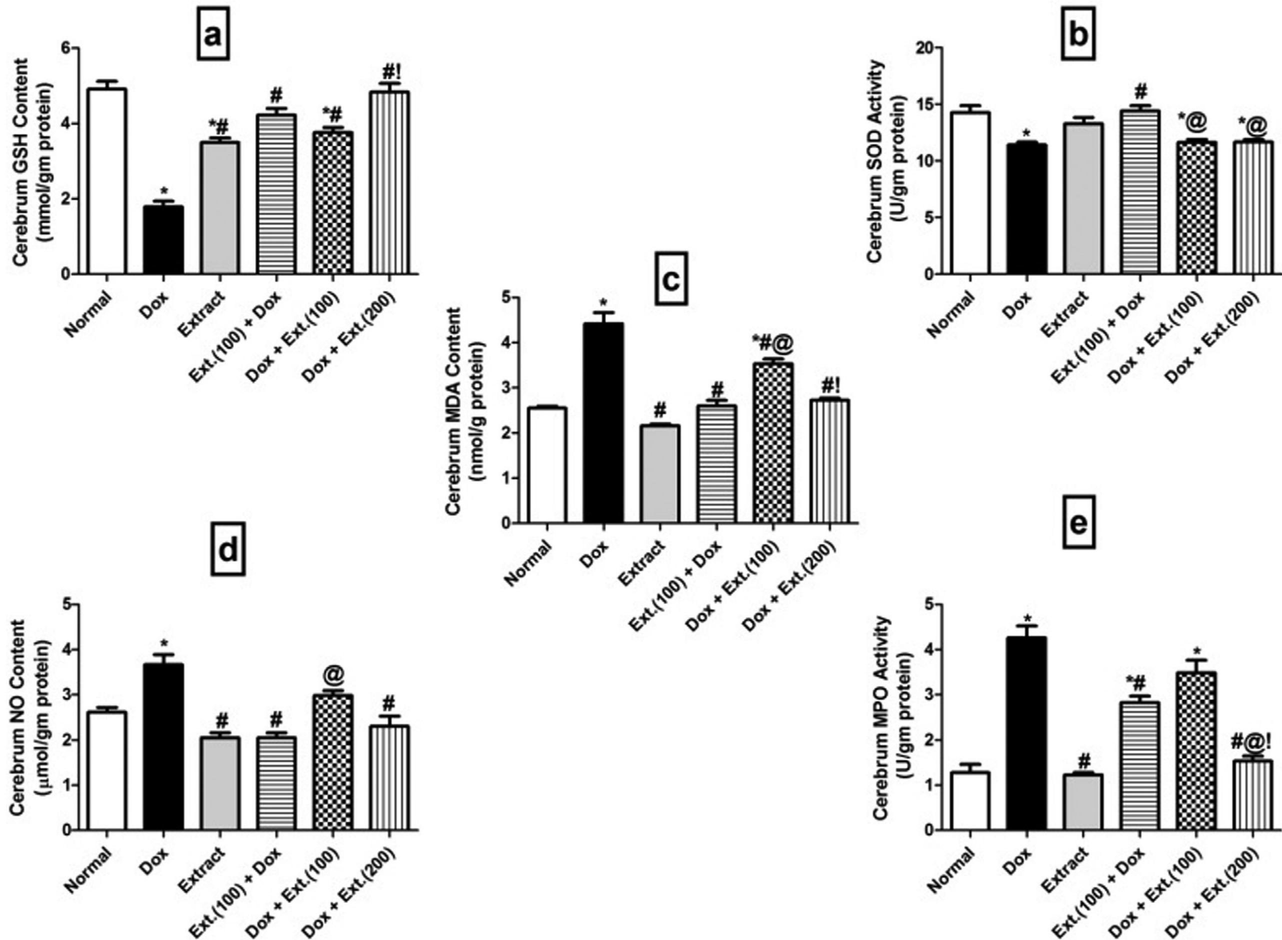
All values of in-vivo results were presented as means ±SEM. Statistical analysis was carried out by one-way analysis of variance followed by Tukey-Kramer multiple comparison tests to calculate the significance of the difference between treatments. *P* value less than 0.05 were considered significant. Statistical analysis was done using GraphPad Prism software (version 5; San Diego, California, USA).

Results

The oxidative and inflammatory markers

As illustrated in Fig. 1, the cerebrum GSH content and SOD activity of the normal group were (4.91 ±0.21 mmol/g and 14.26±0.59 U/g) and were

Figure 1



Neuroprotective effects of TME on (a); cerebrum reduced glutathione (GSH), (b); superoxide dismutase (SOD), (c); malondialdehyde (MDA), (d); nitric oxide (NO), (e); and myeloperoxidase (MPO) in doxorubicin (DOX)-induced neurotoxicity in rats. Each bar represents the mean±SEM. n=4 rats. *Significantly different from the normal group at P value less than 0.05. #Significantly different from control DOX group at P value less than 0.05. @Significantly different from TME_{100}+DOX. !Significantly different from DOX+TME_{100}. TME, *Terminalia muelleri* extract.

significantly decreased in the DOX-treated group by 64% (1.79±0.15 mmol/g) and 20% (11.41±0.25 U/g), respectively, compared with normal rats. Preadministration and coadministration of TME with DOX injection significantly increased both cerebrum GSH and SOD by 136% (4.22±0.17) and 26% (14.41±0.47), respectively as compared with DOX-treated rats. On the other hand, treatment with TME after DOX injection at a dose 100 mg/kg or 200 mg/kg significantly increased GSH by 111% (3.76±0.13), 170% (4.83±0.23), respectively, as compared with DOX-treated rats and significantly decreased SOD by 20% (11.65±0.25), 20% (11.66 ±0.21), respectively, as compared with preadministration and coadministration of TME with DOX injection (14.41±0.47). Treatment with a dose 200 mg/kg of extract significantly increased GSH by 26% (4.83±0.23) as compared with treatment with a small dose of 100 mg/kg of TME (3.76±0.13).

The cerebrum MDA, NO contents, and MPO activity of the normal group were 2.55±0.04 nmol/g, 2.61 ±0.11 μmol/g, 1.28±0.18 U/g, respectively. They were significantly increased in the DOX-treated group by 73% (4.42±0.24), 41% (3.67±0.22), and 233% (4.26 ±0.26), respectively, compared with normal rats.

Compared with DOX-treated rats, the cerebrum MDA, NO, and MPO significantly decreased in groups (preadministration and coadministration) of TME with DOX injection, treatment with a large dose of 200 mg/kg of TME by 41% (2.60±0.12), 40% (2.73±0.04), respectively, by 44% (2.05±0.11), 37% (2.30±0.22), respectively, and by 34% (2.82 ±0.14) and 64% (3.49±0.28). In addition, treatment with a large dose of extract (200 mg/kg) significantly decrease MDA by 23% (2.73±0.04), MPO by 56% (1.53±0.10) as compared with treatment with a small dose of TME (100 mg/kg), (3.53±0.11) and (3.49

±0.28) respectively, and with a significant decrease in MPO by 46% (1.53±0.10), compared with preadministration and coadministration of TME with DOX injection (2.83±0.14). On the contrary, treatments with a small dose of TME (100 mg/kg) significantly increase both MDA and NO by 35% (3.53 ±0.11) and 46% (2.98±0.11), respectively, compared with preadministration and coadministration of TME with DOX injection.

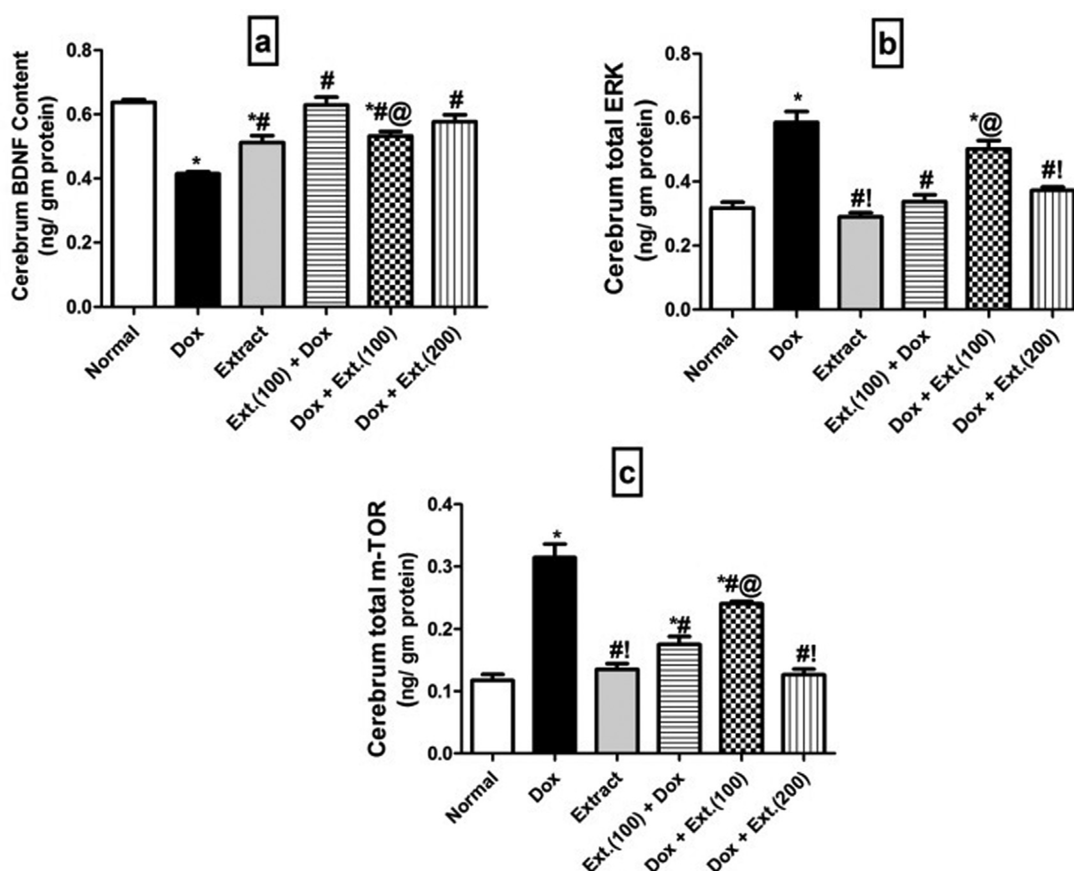
The cerebrum brain-derived neurotrophic factor, extracellular signal-regulated kinase, mammalian target of rapamycin contents

As illustrated in Fig. 2, the cerebrum BDNF, ERK, and m-TOR of the normal group were 0.64±0.008, 0.32±0.01 ng/g, and 0.12±0.009 ng/g, respectively. BDNF significantly decreased in the DOX-treated group by 36% as compared with normal rats (0.41 ±0.006). Preadministration and coadministration of TME with DOX injection markedly increased the reduced cerebrum BDNF by 54% (0.63±0.02) as compared with DOX-treated rats. Also, treatments with a high dose of TME 200 mg/kg significantly increase BDNF by 46% (0.58±0.02), as compared with DOX-treated rats. On the contrary, treatment with a small dose of TME (100 mg/kg) significantly decrease BDNF by 15% (0.53±0.01) as compared with preadministration and coadministration of TME with DOX injection.

Both total ERK and m-TOR were significantly increased in the DOX-treated group by 81% (0.58 ±0.03) and 160% (0.31±0.01), respectively, as compared with normal rats; 0.32±0.02 and 0.12 ±0.009, respectively. Preadministration and coadministration of TME with DOX injection markedly decreased both total ERK and m-TOR by 41% (0.34±0.02) and 44% (0.18±0.01), respectively, as compared with DOX-treated rats.

Similarly, treatments with a high dose of TME 200 mg/kg significantly decrease both total ERK and m-TOR by 36% (0.37±0.01) and 60% (0.13±0.009), respectively, as compared with DOX-treated rats. On the contrary, treatment with a small dose of TME (100 mg/kg) significantly increase both total ERK and

Figure 2



Neuroprotective effects of TME on (a); cerebrum brain-derived neurotrophic factor (BDNF), (b); total extracellular signal-regulated kinase (ERK), (c); and total mammalian target of rapamycin (m-TOR)contents in doxorubicin (DOX)-induced neurotoxicity in rats. Each bar represents the mean±SEM. n=4 rats. *Significantly different from normal group at P value less than 0.05. #Significantly different from control DOX group at P value less than 0.05. @Significantly different from TME_{100}+DOX. !Significantly different from DOX+TME_{100}. TME, *Terminalia muelleri* extract.

m-TOR by 47% (0.50±0.02) and 37% (0.24±0.004) respectively as compared with preadministration and coadministration of TME with DOX injection. As compared with treatment with a small dose of extract (100 mg/kg), treatment with a large dose of 200 mg/kg of TME significantly decrease both total ERK and m-TOR by 26 and 46%, respectively.

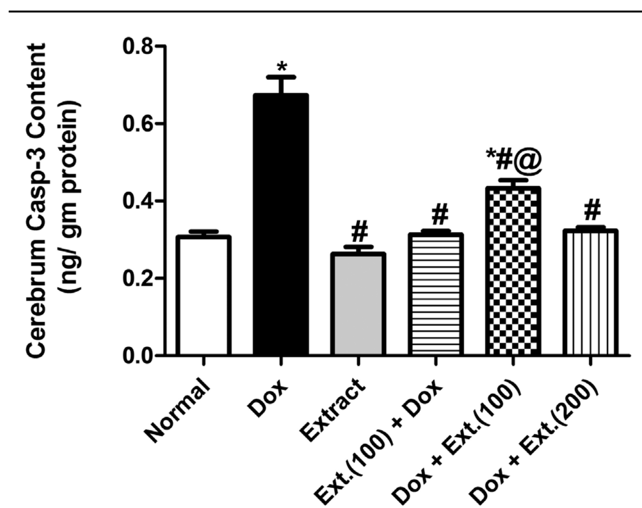
The cerebrum apoptotic marker (caspase-3)

As illustrated in Fig. 3, the cerebrum caspase-3 of the normal group was 0.31±0.01 ng/g. Caspase-3 was significantly increased in the DOX-treated group by 116% (0.67±0.04) as compared with normal rats. On the contrary, pre- and coadministration of TME with DOX injection and treatment with a large dose of TME 200 mg/kg markedly decreased the elevated caspase-3 content to 56% (0.31±0.008) and 54% (0.32±0.008), respectively, as compared with DOX-treated rats. In addition, treatment with a small dose of TME (100 mg/kg) significantly increased caspase-3 by 39% (0.43±0.02) as compared with pre- and coadministration of TME with DOX injection.

The cerebrum acetylcholinesterase activity

As illustrated in Table 1, AChE was significantly increased in the DOX-treated group by 50% as compared with normal rats. In contrast, preadministration and coadministration of TME with DOX injection and treatment with a large dose of TME 200 mg/kg markedly decreased the elevated AChE content to 25 and 35%, respectively, as compared with DOX-treated rats. Similarly, treatment with a high dose of TME 200 mg/kg significantly decreased AChE by 33% as compared with treatment with a small dose of TME.

Figure 3



Neuroprotective effects of TME on cerebrum brain caspase-3 (Casp-3) contents in doxorubicin (DOX)-induced neurotoxicity in rats. Each bar represents the mean±SEM. *n*=4 rats. *Significantly different from normal group at *P* value less than 0.05. #Significantly different from control DOX group at *P* value less than 0.05. @Significantly different from TME_{100}+DOX. TME, *Terminalia muelleri* extract.

Histopathological examinations

Figures 4–6.

Discussion

The brain, with a high content of polyunsaturated fatty acids and high oxygen demand, is a very complicated and sensitive organ, which is highly affected by chemotherapeutic drugs that are used in the treatment of cancer [39–43].

In fact, DOX undergo redox cycling and can generate high levels of ROS and inflammatory mediators by the accumulation of the products of lipid peroxidation, NO, MPO, and depletion of antioxidant status indices and stimulation of cell apoptosis in the brain [44–48]. In agreement with the findings of several studies [49–51], our results revealed the change in the levels of MDA, NO contents, and MPO activity that correlated with decreasing endogenous antioxidants such as SOD activity and GSH contents in the DOX-treated group as compared with the control group, which can be interpreted on the basis of their exhaustion to balance the elevation in ROS production [52]. Increasing NO may be a result of rising circulating levels of TNF-α in the brain and subsequent increase of inducible NO synthase expression [53].

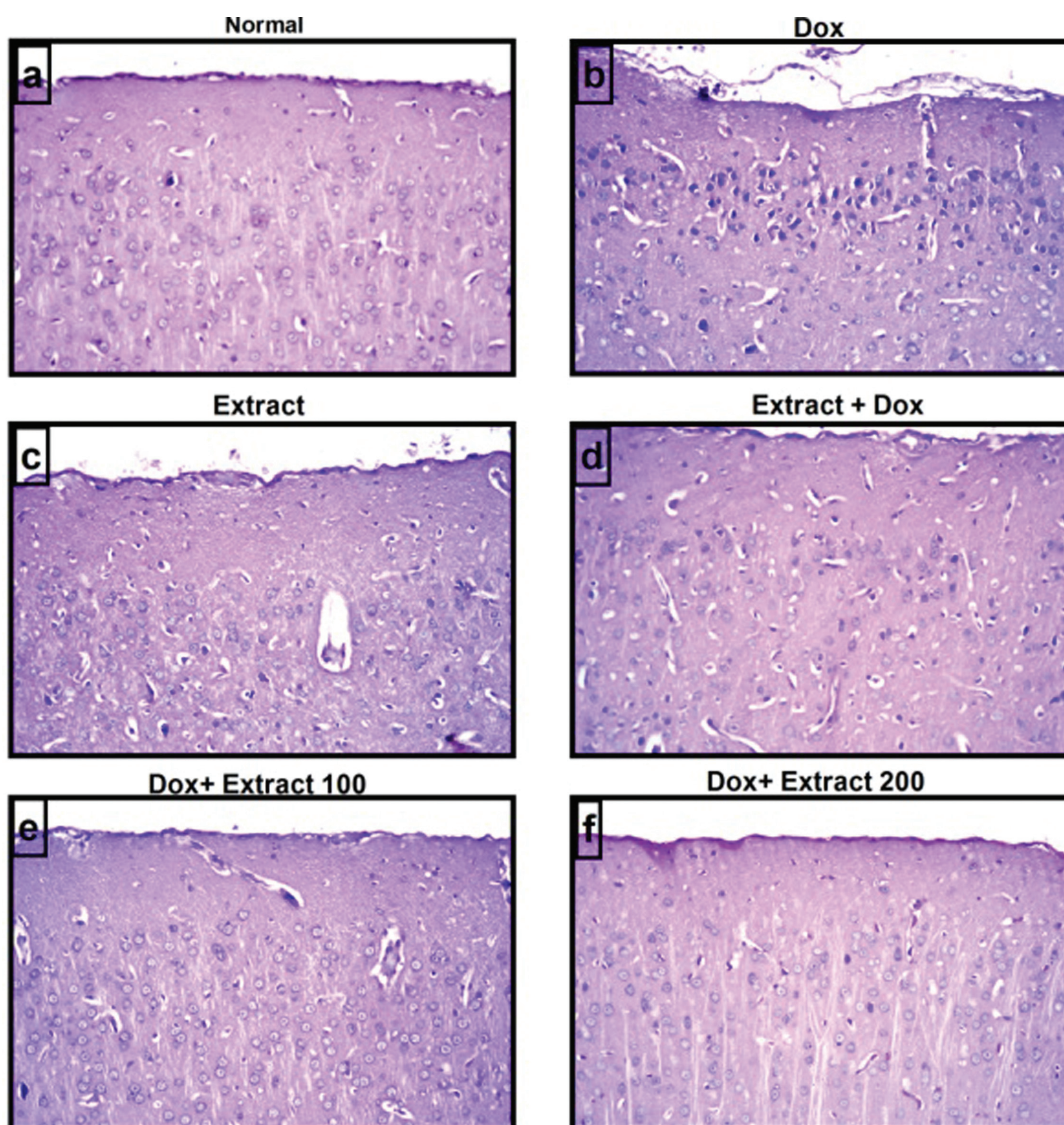
Noteworthy, treatment with TME improved the levels of oxidative stress and inflammatory biomarkers, where this improvement is more pronounced in the group treated with 200 mg of TME after DOX injection. These findings were consolidated by the alterations of histopathological observations. Herein, treatment with TME lowered MDA, increasing GSH and SOD

Table 1 Neuroprotective effect of *Terminalia muelleri* extract on cerebrum acetylcholinesterase activity in doxorubicin-induced neurotoxicity in rats

Groups	AChE activity (U/g protein)
Normal	7.69±0.50
DOX	11.53±0.61*
Extract	8.23±0.70#
Ext.100 +DOX	8.64±0.53#
DOX+Ext.100	11.28±0.43*@
DOX+Ext.200	7.54±0.19#!

Each value represents the mean±SEM. AChE, acetylcholinesterase; DOX, doxorubicin; TME, *Terminalia muelleri* extract. n=4 rats. *Significantly different from normal group at *P* value less than 0.05. #Significantly different from control DOX group at *P* value less than 0.05. @Significantly different from TME_{100}+DOX. !Significantly different from DOX+TME_{100}.

Figure 4



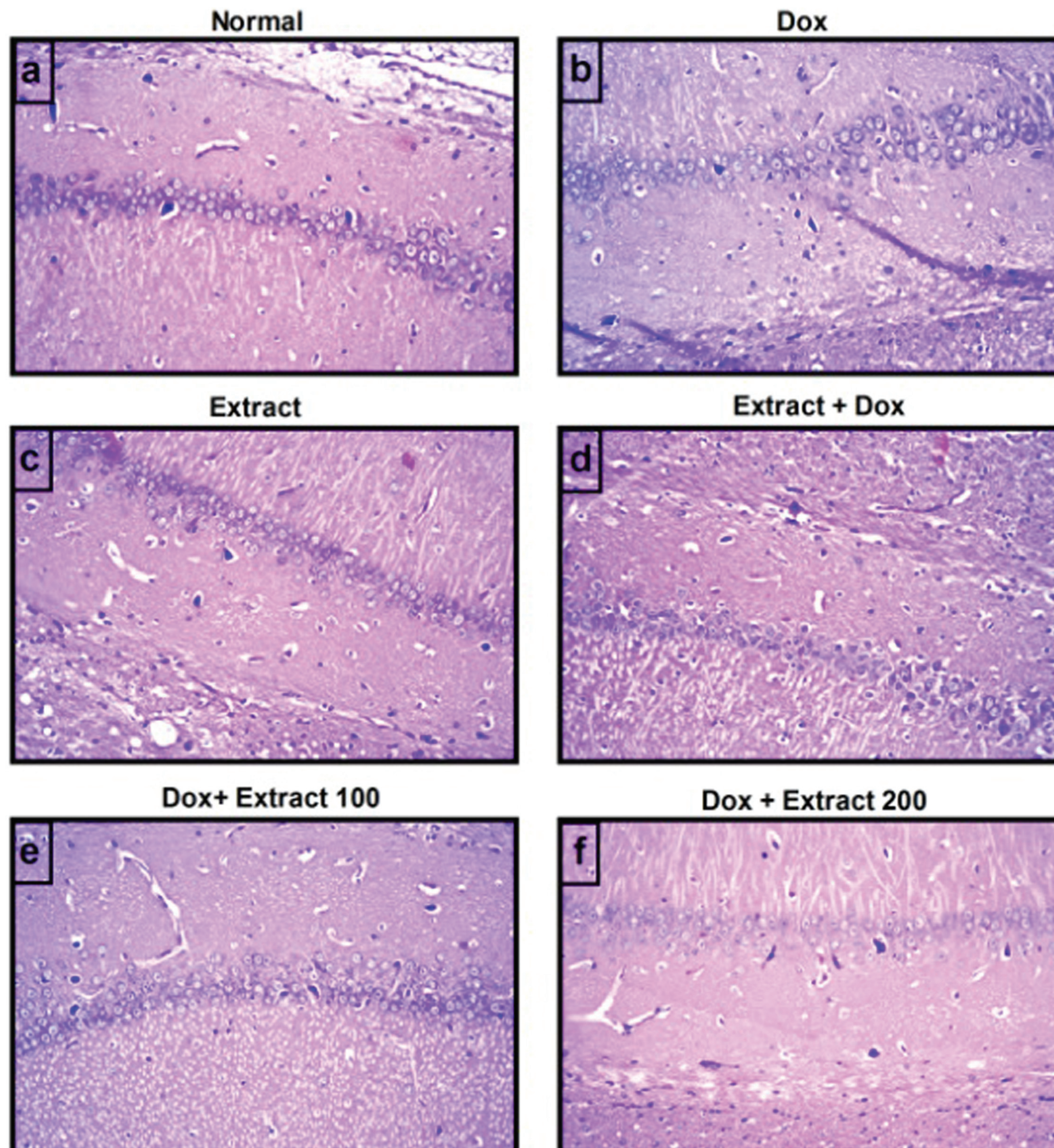
Photomicrographs of rat cerebral cortex sections stained with H&E: groups 1, 3 showed no histopathological alterations, sections of rat from the doxorubicin (DOX)-treated group 2 showed nuclear pyknosis and degeneration in some neurons (b). Sections of rat from groups 4, 5, 6 showed normal histological appearance of neurons (d, e, f, respectively) (H&E, ×40).

activity, decreasing NO and MPO levels as compared with the DOX-treated group, which may be attributed to the inhibition of cyclooxygenase-2 and subsequent inhibition of prostaglandin synthesis [29]. Our results are in line with the studies of Fahmy *et al.* [27], El-Kashak *et al.* [54], and Ahmed *et al.* [55]. Antioxidant and radical scavenging activity of TME is essentially associated with the presence of polyphenols such as flavonoids, gallic acid and ellagic acid [27], which can act as singlet oxygen scavengers, hydrogen atom donors, and reducing agents [56]. Flavonoids, ellagic acid, and gallic acid are well known to have antioxidant properties and anti-inflammatory activity [57,58] and may be responsible for significant decrease in MDA and increase in the endogenous antioxidants in the TME-treated groups.

Oxidative stress can change the cholinergic transmission by suppressing muscarinic cholinergic receptors in the brain [59]. In our study, AChE is increased in the DOX-treated group compared with the control group, which was reversed by the administration of TME [28,50,60]. This may be attributed to the presence of gallic acid and ellagic acids, which strongly inhibited the AChE activity [61,62] and this could be due to their potent antioxidant activity.

Our results were consistent with the study by Risk *et al.* [42], who reported that DOX administration triggered apoptosis in brain tissue through depolarization of the mitochondrial membrane, resulting in the release of cytochrome C into the cytosol leading to a set of

Figure 5

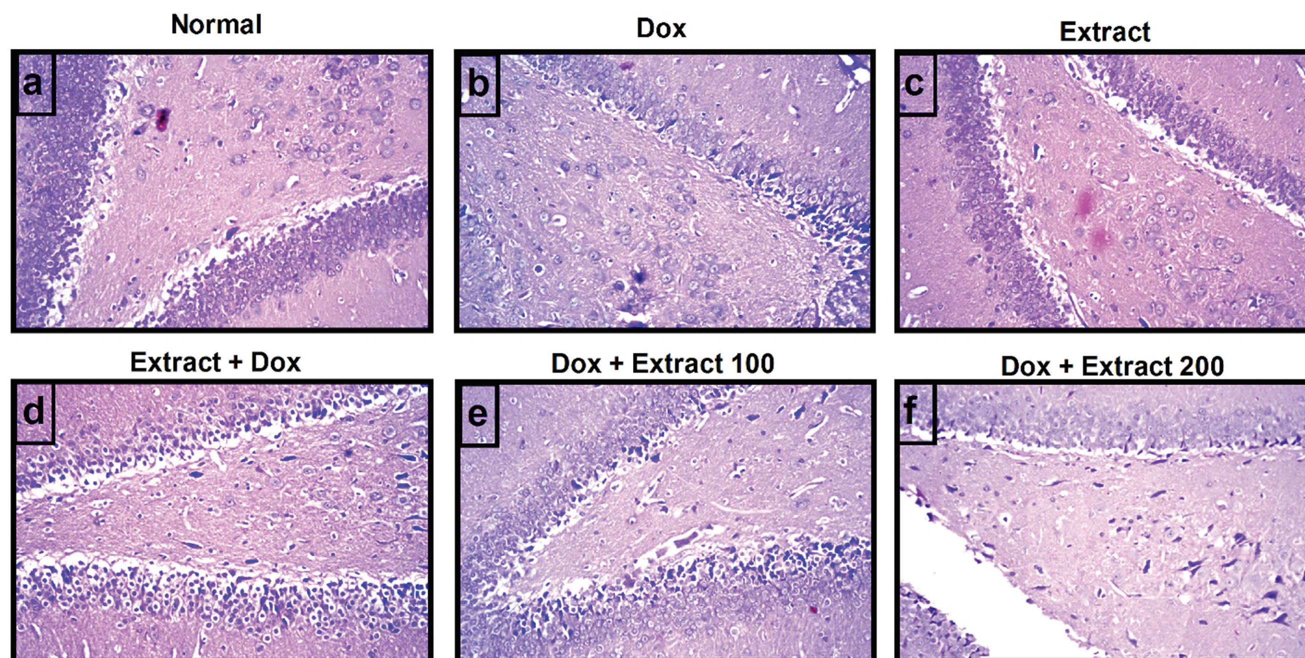


Photomicrographs of rat subiculum in hippocampus sections stained with H&E: groups 1, 3 showed normal histological structure of neurons, sections of rat from the doxorubicin (DOX)-treated group 2 showed nuclear pyknosis in a number of neurons (b). Sections of rat from groups 4, 5, 6 showed normal histological appearance of the neurons (d, e, f, respectively) (H&E ×40).

apoptotic reactions that resulted in programmed cell death [63,64]. Treatment with TME significantly decreased caspase-3 level by downregulation of the protein expression and activity of caspase-3 [65]. In addition, gallic acid and ellagic acid can protect against mitochondrial dysfunction by inhibiting cytochrome p450 enzyme [66].

During exposure to oxidative stress, BDNF level was reduced leading to neurodegeneration which results in a cognitive impairment [67]. In the current study, DOX administration reduced BDNF level as per the results of Park *et al.* [68]; this reduction in BDNF level may be attributed to the decrease of glutamate clearance after DOX administration where glutamate could diffuse to and bind with N-methyl-D-aspartate receptors, and activation of extrasynaptic N-methyl-D-aspartate receptor causes inhibition of BDNF synthesis, leading to a loss in synaptic plasticity and an elevation in neuronal apoptosis [63,69].The activation of ERK is mostly associated with survival signals but with several chemotherapeutic drugs, its activation promotes apoptosis [64]. The results of our study showed that the administration of DOX increased total both ERK and m-TOR. Consistent with our study, Liao *et al.* [49] also found that the rats treated with DOX show significantly suppressed pathway of ERK phosphorylation through suppression of NRG1/ErbB signaling and impairment of the downstream Akt and ERK

Figure 6



Photomicrographs of rat fascia dentate and hilus in hippocampus sections stained with H&E: groups 1 and 3 showed normal histological structure of neurons, sections of rat from doxorubicin (DOX)-treated; group 2 showed nuclear pyknosis and degeneration in the majority of the neurons (b). Sections of rat from groups 4, 5, and 6 showed nuclear pyknosis and degeneration in some neurons (d, e, f) (H&E ×40).

pathway in rats, which raised a stimulating possibility for the involvement of the NRG1/ErbB pathway in DOX-induced neurotoxicity.

The m-TOR is an intracellular protein kinase that functions as an energy and nutrient sensor in the cellular microenvironment of neurons. Modulation of m-TOR is vital when nutrient and energy sources become limited. Hypoxia, traumatic brain injury, cellular energy states, and growth factors all regulate the phosphorylation and total levels of m-TOR in cells [70]. Phosphorylation of m-TOR at the serine 2448 site allows for the formation of m-TOR complex 1. Under normal conditions, when m-TOR complex 1 is activated, cellular growth and metabolism, protein synthesis, and lipid synthesis are stimulated [71,72]. Our results in the same line with the preliminary results of Li *et al.* [73] showed DOX-inhibited cell growth, induced apoptosis, and decreased the expression of m-TOR in K562 cells.

In this context, treatment with TME in our study obviously normalized the levels of BDNF, ERK, and m-TOR level caused by DOX probably due to the presence of bioactive compounds as gallic and ellagic acids [74], flavonoids and polyphenolic compounds through their antioxidative activity, which may be involved in protecting against stress-related neurodegeneration and restoration of BDNF decline levels, which may be due to the presence of neurotrophins and growth factors such as the nerve growth factor [75].

Similar histopathological changes as nuclear pyknosis and degeneration in some neurons in the DOX group in most of the neurons of the cortex, subiculum, fascia dentata, and hilus of the hippocampus combined with focal hemorrhage were reported by other studies that induced chemobrain in laboratory animals by DOX [76,77].

Conclusion

Nevertheless, this study opens up a new window of understanding the valuable role of TME in protecting or treating the neurodamaging effects induced by DOX through suppressing oxidative stress, inflammation, apoptosis, and autophagy in the brain of rats. Thus, the approach of TME could be applied in future to treat or prevent DOX-induced neurotoxicity in cancer therapy.

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experiments. M.A.M. analyzed data. S.M.A. and M. A.M. wrote the manuscript. S.M.A. and M.A.M. read and approved the manuscript, and all data were generated in-house and that no paper mill was used.

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Conflicts of interest
There are no conflicts of interest.

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