Effect of Testosterone on the Cerebral Cortex Bcl-2 Expression and Oxidative Stress in a Rat Model of Vascular Dementia

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Abstract

Objectives to investigate the effect of testosterone on the cerebral cortex Bcl-2 expression and oxidative stress in a rat model of vascular dementia (VD). *Methods* Forty-eight Sprague-Dawley adult male rats were divided into four groups (n=12, each) as follows (i) untreated control, (ii) rats exposed to surgical permanent bilateral occlusion of the common carotid arteries (BCCAO) leading to chronic cerebral hypoperfusion, (iii) rats exposed to BCCAO then received testosterone (0.5 mg/ kg S.C., three times/ week, S.C.) (BCCAO + Tes) for 4 weeks and (iv) rats exposed to BCCAO then received donepezil (3 mg/kg/ day; i.p.) (BCCAO + DON) for 4 weeks. Levels of acetylcholine and norepinephrine in the cerebral cortex were measured. Furthermore, the expression of Bcl-2 in the cerebral cortex and malondialdehyde (MDA), non-enzymatic (reduced glutathione, GSH) and enzymatic [superoxide dismutase (SOD) and catalase (CAT)] anti-oxidants were determined. Histopathological studies of the cerebral cortex were performed. *Results* BCCAO decreased the expression of Bcl-2 as well as the central level of acetylcholine and norepinephrine as compared to control rats. Also, increased the cerebral cortex level of MDA and decreased the enzymatic and non-enzymatic antioxidant. Treatment with testosterone and donepezil increased acetylcholine and norepinephrine significantly when compared with BCCAO rats. Testosterone significantly increased the expression of Bcl-2 and antioxidant system with significant reduction in MDA. *Conclusion* Testosterone might employ neuroprotective effects through increasing the expression of Bcl-2 as well as ameliorating the oxidative stress induced by BCCAO in rat model of DA.

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Disorders of the cerebral circulation are the causes of numerous neurological and psychiatric illnesses. Reduction in regional cerebral blood flow (CBF) compromises memory processes and contributes towards the development and progression of dementia. The association of decreased CBF, particularly in the temporal and parietal cortices, with Alzheimer's disease (AD) has been firmly established.[1-3] Cerebral ischemia is associated with decreased partial pressure of oxygen (O_2) causes a reduction in available cellular O_2, which is the ultimate electron acceptor in the electron transport chain.[4] Due to low levels of O_2, the electron accumulation is higher, and more electrons attack the ground state of available O_2 to form superoxide anion (O_2^-); in turn, hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) are formed by chain reaction. The hypoxia-induced generation of reactive oxidative stress (ROS) can cause protein oxidation, DNA and RNA oxidation, lipid peroxidation and neuronal dysfunction or death.[5] The brain is sensitive to oxidative stress due to (i) an abundant presence of polyunsaturated fatty acid, (ii) deficient antioxidant defence, (iii) a high rate of O_2 utilisation due to the high metabolic rate and (iv) a high content of transition metals such as copper and iron in several regions, which

could lead to the formation of hydroxyl radicals via the Fenton reaction.[6]

Testosterone and diahydroepiadrosteinedione (DHEA) actions are mediated in part via activation of androgen receptors (AR), which are localized in many brain areas, including regions important for learning and memory such as the hippocampus and amygdala. Advantageous actions of androgens in the brain include stimulation of neuronal differentiation, maintenance of neuronal morphology density and promotion of synaptic density. For example, studies of the hippocampus in male rats showed a significant decreased in the density of spine synapses following gonadectomy (GDX), an effect reversed by replacement with either testosterone or dihydrotestosterone (DHT). Indeed, castration of male rats induces oxidative stress in the rat prostate by significantly upregulating ROS-generating NADPH oxidases and down-regulating ROS-detoxifying enzymes.[7,8] Moreover, Tunez et al.[9] has reported that testosterone (0.5 mg/kg subcutaneously (s.c.) for 8 days) reduced the oxidative stress and cell damage in striatum of ovariectomized rats.Donepezil is one of the three currently approved acetylcholine esterase inhibitors for treating AD symptoms delaying the decline in cognitive function.[10]

The founder of this family, the *BCL-2* proto-oncogene, was discovered at the chromosomal breakpoint that bearing human B-cell lymphomas. Bcl-2 family of proteins

has expanded significantly and includes both pro- as well as anti-apoptotic molecules.BCL-2 family members possess up to four conservedBCL-2 homology (BH) domains designated BH1, BH2,BH3, and BH4, which correspond to a-helical segments[11]. It is well known that Bcl-2 protects cells from various stresses such as ionizing radiation exposure[12], chemotherapeutic agent treatment[13], ischemia/reperfusion injury[14], and serum and growth factor withdrawal.[15] It, also, facilitates recovery from DNA damage after oxidative stress.[16] Although the mechanism of protection by Bcl-2 remains still obscure, the protective effect may result from a reduction in the formation of reactive oxygen species[17], the inhibition of cytochrome c release from the mitochondria[18] and the upregulation of the cellular glutathione pool.[19] These protective effects result in inhibition of apoptotic cell death. This experimental study was designed to investigate the protective effect of exogenous subcutaneous (s.c.) administration of testosterone, three times per for 4weeks 4) and intraperitoneal (i.p.) administration of donepezil 3 mg/kg/ day; for 4 weeks on the cerebral cortex Bcl-2 expression and oxidative stress markers in brain of rat model of vascular dementia.

MATERIALS AND METHODS

Experimental Animals

The animals employed in this experimental study comprised of 48 male adult Sprague–Dawley rats weighing 170-200 g, aged from 7-8 weeks. All rats were bred and housed in the research center of Mansoura Faculty of Medicine at a temperature of 23 ± 1 °C, and a 12h light: 12h dark cycle. All rats had a free access to tap water and fed standard laboratory chow. All experimental procedures were approved by the medical research ethical committee at Mansoura Faculty of Medicine and according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

Experimental design

After 1 week of acclimatization to the laboratory environment, the animals were randomly allocated into four groups (each contained12 rats) as follows: 1) Normal rats (control group): consisted of healthy rats not exposed to bilateral common carotid artery occlusion.(BCCAO) 2) Rats exposed to bilateral BCCAO. 3) Rats exposed to BCCAO and treated with testosterone propionate (0.5 mg/ kg dissolved in 0.1 mL of sesame oil) S.C., three times per week (Meydan et al. 2010), (BCCAO + Tes) for 4 weeks 4) Rats exposed to BCCAO and treated with donepezil 3 mg/kg/ day; i.p. (BCCAO + DON) for 4weeks.

Bilateral common carotid occlusion

Bilateral common carotid arteries were occluded as previously described[20] under ketamine hydrochloride (50 mg/kg, i.m.) andxylazine (5 mg/kg, i.m.) anesthetics. To prevent respiratory distress, the rats were also administered atropine sulfate (0.1 mg/kg, i.m.). The common carotid arteries were carefully separated from surrounding tissues, including the vagus nerve and ligated with coated Vicryl (R) Plusantibacterial/Polyglactin 910 3/0 absorbable surgical suture (Ethicon, Johnson & Johnson, UK), approximately 1 cm inferior to the origin of the external carotid artery. The control rats were subjected to the same surgical procedure without occlusion of the arteries.

Chemicals:

Testosterone propionate and donepezil (AChase inhibitor) were obtained from Sigma Aldrich.

Sample preparation and biochemical evaluations

The rats were euthanized after the end of the treatment. The brain was quickly removed from the skull; the hippocampus and the rest of the brain were dissected. Tissue samples were immediately frozen in liquid nitrogen and kept at -70°C until assayed. The cortex norepinephrine and acetylcholine concentrations were measured by ELISA kits supplied from Abnova.. Assessment of lipid peroxide, enzymatic and non-enzymatic antioxidants, and vitamin E

in cerebral cortex: The cerebral cortex was removed and rinsed with phosphate-buffered saline (pH 7.4) to remove any red blood cells or clots. Next, the tissue was homogenised in 5–10 mL of cold 20 mmol/L HEPES buffer (pH 7.2) containing 1 mmol/L EGTA, 210 mmol/L mannitol, and 70 mmol/L sucrose per gram of tissue. The homogenates were centrifuged at 15000 r/min (18 000×g) for 15 min at 4 °C, and the supernatant was removed and stored at –80 °C until further analysis.

Biochemical investigations

In the cerebral cortex tissue homogenate, the levels of glutathione (GSH) (Cat. No. 703002; Cayman Chemical, Ann Arbor, Mich., USA), superoxide dismutase (SOD) (Cat. No. 706002, Cayman Chemical, Ann Arbor, Mich., USA), and catalase (CAT) (Cat. No. NWK-CAT01, Northwest Life Science Specialties, Vancouver, Wash., USA) were measured according to the manufacturer's instructions. In addition, malnaldehyde (MDA) was analyzed in the brain homogenateby measuring the production of thiobarbituric acid-reactive substances (TBARS) with a TBARS assay kit (Cat. No. 10009055, Cayman Chemical, Ann Arbor, Mich., USA). Also, norepinephrine levels in the tissue homogenates were quantified using the ELISA technique with commercially available kits (CatCombi ELISA; RE59242, DRG international. Inc. for NE Biocompare

company for Ach, USA). The reactions were read using an ELISA microplate reader (TLACOSR 496; Mabaret-Alasafra, Alexandria, Egypt). The concentrations of epinephrine and norepinephrine were measured in ng/gm tissues.

Total RNA extraction from the hippocampus of rats:

Total RNA was extracted from the hippocampus, the cortical tissue was shock freeze by liquid nitrogen and used immediately for RNA extraction using TriFast^{TM} reagent (PeqLab. Biotechnologie GmbH, Carl-Thiersch St. 2B 91052 Erlongen, Germany, Cat. No. 30-2010) according to the manufacturer's instructions. The remaining DNA was removed by digestion with DNase I (Sigma).The concentration of isolated RNA was determined spectrophotometrically by measuring the optical density (OD) at 260 nm (Jenway, Genova Model, UK). 10µl of each sample was added to 990µl of DEPC treated water and quantified by measuring the absorbance at 260nm as RNA yield (µg/ml) = A260 X 40 X 100 (dilution factor). The purity of RNA was determined by gel electrophoresis through formaldehyde agarose gel electrophoresis and ethidium bromide staining to show 2 sharp purified bands, these two bands represented 28S and 18S ribosomal RNA.

Semiquantitative real timePCRor extracted RNA:

RT-PCR was performed using Ready-to-Go RT-PCR beads for first cDNA synthesis and PCR reaction provided by Amersham Biosciences, England. Cat. No. 27-9266-01.Ready-to-Go RT-PCR beads utilize Moloney Murine leukemia virus (M-MuLV) reverse transcriptase and Taq polymerase to generate PCR product from RNA template. Each bead is optimized to allow the first strand cDNA synthesis and PCR reaction to proceed sequentially as a single tube, single step reaction. The reaction passed as follow:

a) Synthesis of cDNA:

The followings were added to each tube containing the beads: 2 µl of first strand primer, provided by the kit, 3 µl containing 30 pmol of PCR gene-specific primer (sense), 3µl containing 30 pmol of PCR gene-specific primer (anti-sense), 25 µl of total template RNA containing 1ug and 17µl of DEPC-treated water to obtain a total volume of 50 µl. One tube was prepared as a negative control reaction to test for DNA contamination.The dehydrated bead (without template and primers) was incubated at 95°C for 10 minutes to inactivate the M-MuLV reverse transcriptase. 50 ul mineral oil were added to overlay the reaction. The reactions were transferred to the thermal cycler and incubated at 40°C for 30 minutes for synthesis of cDNA followed by incubation at 95°C for 5 minutes to inactivate the reverse transcriptase and completely denature the template.

b) Gene specific primers used were:

Gene specific primers were purchased from Biolegio. BV, PO Box 91, 5600 AB Nijmegen, Netherlands.

Gene	Primer
Bcl-2	Forward 5`--TGG GAT GCC TTT GTG GAA CTA T-` Reverse 5`--AGA GAC AGC CAG GAG AAATCA AAC--3`.
Internal housekeeping (control) gene (β-actin)	Forward 5`- CACAGCTAGAGGGAAATCG -3`. Reverse5`- 5'CACC AGAGTAGTTGCGCTC -3`.

A)   Amplification of cDNA by PCR:

Thermal cycling reaction was performed using thermal cycler (TECHEN TC-312,Model   FTC3102D,Barloworld Scientific Ltd. Stone,StaffordShire,st 150 SA,UK) with the following program:

	Bcl-2	β-actin (internal control-Housekeeping gene)
Initial denaturation	94◦C for 5 minutes	94◦C for 5 minutes
Cycles		
Number	30	25
-denaturation	94 ◦C for 1 minute	94 ◦C for 1 minute
-primer annealing	55◦C for 1 minute	55◦C for 1 minute
-Extension	72 ◦C for 1 minute	72 ◦C for 1 minute
Final extension	68 ◦C for 7 minutes	72 ◦C for 7 minutes
Product size	217 bp	110

c)   Detection   of   amplified   RT-PCR products:

For semiquantitative RT-PCR, the products of amplification were subjected to agarose gel electrophoresis using 2% agarose stained with ethidium bromide and visualized via light ultraviolet transilluminator (Model TUV-20, OWI. Scientific, Inc. 800 242-5560) and photographed under fixed conditions (the distance, the light and the zoom).The results photos were analyzed with scion image ® release Alpha 4.0.3.2. Software for windows ® which performs bands detection and conversion to peaks. Area under each peak were calculated in square pixels and used for quantification. Gene expression levels were determined by calculating the ratio between the square pixel values of the target gene in relation to the internal housekeeping control gene (β-actin). Negative control tubes showed no PCR products indicating that all reagents were   free   from   target   sequence contamination.

Histological examination

The brain tissue was fixed in 10 % formalin for one week, washed in running tap water for 24 h and dehydrated in an ascending series of ethanol (50–90 %), followed by absolute alcohol. The samples were cleared in xylene and immersed in a mixture of xylene and paraffin at 60 ℃. The tissue was then transferred to pure paraffin wax of the melting point 58 ℃ and then mounted in blocks and left at 4 ℃. The paraffin blocks were   sectioned   on   a   microtome   at   a thickness of 5 μm and mounted on clean glass slides and left in the oven at 40 ℃ to dryness. The slides were deparaffinized in xylene and then immersed in descending series of ethanol (90–50%). The ordinary haematoxylin and eosin stain were used to stain the slides.

Statistical   analysis

  Statistical   analysis   was   performed   by

using GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA). The data were analyzed by One-way ANOVA, followed by the Tukey Multiple Comparison Test for parametric data. Pearson correlation was made between Bcl-2 and measured parameters. P value of 0.05 or less was considered significant.

RESULTS

Table (1) showed a significant increase in the cerebral cortex MDA (P<0.05) but significant decreased in total antioxidant capacity in the cerebral cortex homogenate (GSH, SOD and CAT) (P<0.05 for all) in BCCAO compared to control rats. On the other hand, testosterone treatment significantly decreased MDA (P<0.05) but significantly increased (P<0.05) the antioxidant enzymes as compared to BCCAO group. Although testosterone changed the MDA and antioxidant enzymes, the changes were still different from the control. The MDA was still significantly elevated and the antioxidant enzymes were still significantly less than the control.Donepezil treatment did not insignificantly change the oxidative stress parameters when compared with BCCAO rats (P>0.05) while, it was significantly higher than the control and BCCAO with testosterone treatment.

Table (1): Effect of testosterone on cerebral cortex lipid peroxidation (MDA) and antioxidants (GSH, SOD and CAT) in rats with BCCAO.

	Control	BCCAO	BCCAO + Tes	BCCAO +DON
MDA (nmol/g tissue)	28.40 ± 1.70	59.70 ± 3.48	44.56 ± 2.91^{ab}	55.30 ± 3.23^{ac}
GSH (µmol/g tissue)	1.99 ± 0.14	0.85 ± 0.09^{a}	1.65 ± 0.08^{ab}	0.92 ± 0.12^{ac}
SOD (µg/g tissue)	39.70 ± 2.30	24.90 ± 1.85^{a}	33.60 ± 2.02^{ab}	26.94 ± 1.34^{ac}
CAT (µmol /g tissue)	188.00 ± 8.72	95.00 ± 6.64^{a}	128.00 ± 7.39^{ab}	99.43 ± 5.29^{ac}

Values were expressed as mean ± SD of eight rats in each group MDA= malondialdehyde, GSH= reduced glutathione, CAT= catalase, SOD= superoxide dismutase, BCCAO = Bilateral common carotid artery occlusion, Tes = Testosterone and DON= Donepezil. a p< 0.05 significant as compared to control rats. b p<0.05 significant as compared to BCCAO rats.c p<0.05 significant as compared to BCCAO+ Tes

Fig. (1) showed the cerebral cortex level of acetylcholine and norepinephrine. BCCAO resulted in a significant decreased in the cerebral cortex level of a both acetylcholine and norepinephrine when compared to control rats (P<0.05 for both). Testosterone supplementation after BCCAO produced a significant increase in both acetylcholine and norepinephrine cerebral cortex levels when compared to the BCCAO rats (P<0.05 for both) but still lower than the control rats. In donepezil treatment after BCCAO, cerebral cortex level of acetylcholine and norepinephrine were significant increased compared with BCCAO rats(P <0.05 for both) but insignificantly changed of acetylcholine when compared to testosterone treated rats (P >0.05). Also donepezil treatment after BCCAO significantly P <0.05 reduced both acetylcholine and norepinephrine.

(a)

(b)
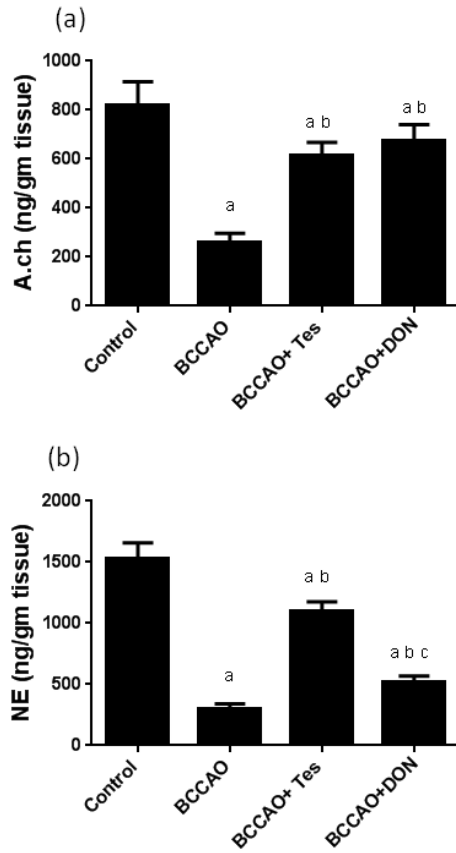
Fig. (1): The central level of **(a)** acetycholine and **(b)** norepinephrine (ng/ mg tissue) in the hippocampus. All data were expressed as mean ± S.D. (n=10)

a: p<0.05 as compared with the control group.

b: p<0.05 as compared with the BCCAO non treated group.

c: p<0.05 as compared with the BCCAO + testosterone treated group.

Figs. (2a and b) showed the expression of Bcl-2 in the experimental groups. Bcl-2 expression in cerebral cortex in BCCAO group was significantly decreased compared to the control rats (P <0.05); significantly increased BCCAO + testosterone treated group, but insignificant changed in BCCAO + donepezil treatment group (P >0.05).

(a)

(b)
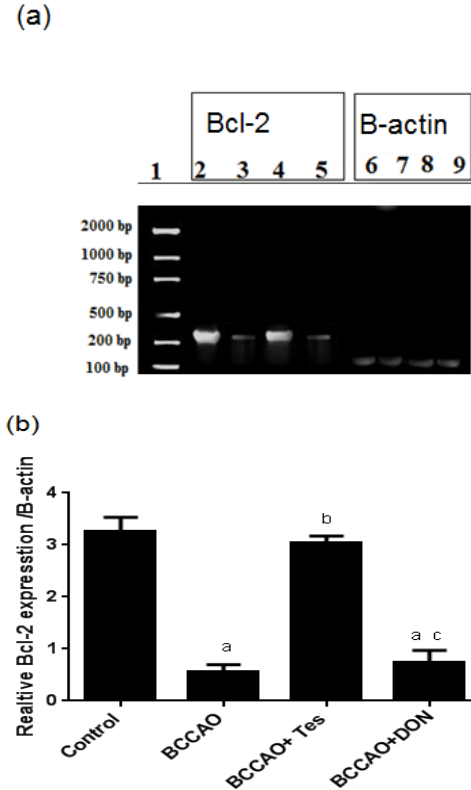
Fig. (2) a: Semiquantitative reverse transcriptase PCR products of cerebral cortex mRNA of Bcl-2 and β-actin (housekeeping gene). The RT-PCR products, obtained from all groups were separated by 2% agarose gel electrophoresis containing 100 ng/ml ethidium bromide. **1:** Ladder. **2 and 6**: control group. **3 and 7:** BCCAO non treated rats. **4 and 8:**BCCAO + testosterone treatment. **5 and 9:** BCCAO + donepezil treatment. **b:** relative BCL-2 expression to housekeeping (B-actin) gene expression in the cerebral cortex.

All data were expressed as mean ± S.D. (n=10);

a: p<0.05 as compared with the control group.

b: p<0.05 as compared with the BCCAO non treated group.

c: p<0.05 as compared with the BCCAO + testosterone treated group.

Table (2) showed significant negative e correlations between BCL2 expression in the cerebral cortex with the MDA level in the homogenate of the cerebral cortex in different groups. At the same time BCL2 expression showed positive correlations with

GSH, SOD and catalase enzymes in the homogenate of the cerebral cortex in all groups except GSH and SOD in control group which showed non-significant positive correlation with BCL2 expression.

Table (2): Correlations between BCL2 expression and other studied parameters in different groups

|		Control	BCCAO	BCCAO + Tes	BCCAO +DON
MDA	r	-0.655	-0.911	- 0.878	- 0.812
	p	0.0210	0.000	0.000	0.001
GSH	r	0.450	0.832	0.822	0.754
	p	0.052	0.000	0.000	0.001
SOD	r	0.357	0.757	0.801	0.649
	p	0.281	0.003	0.002	0.023
CAT	r	0.679	0.892	0.833	0.739
	p	0.005	0.000	0.001	0.010

Pearson correlation between BCL2 and MDA, SOD, CAT. MDA= malondialdehyde, GSH= reduced glutathione, CAT= catalase, SOD= superoxide dismutase, BCCAO = Bilateral common carotid artery occlusion, Tes = Testosterone and DON= Donepezil. Significant $p \leq 0.05$

Fig. (3) showed the histopathological changes in of the cerebral cortices of the control and experimental rats (original magnification X400). Figure (3A) section) showed intact neurons and well-preserved cell density in cerebral cortex of control rats. Figure (3B) showed neuronal loss with ischemic changes (pyknotic nuclei and cellular necrosis in some areas, indicative of cerebral infarction and necrosis) in cerebral cortex of BCCAO rats. Figure (3C) showed more or less intact neurons with intact chromatin in cerebral cortex tissue of BCCAO + testosterone treated rats. Figure

(3D) showed the same ischemic changes but less than the BCCAO rats in cerebral cortex tissue of BCCAO + donepezil treated rats.

DISCUSSION

The present study demonstrated that testosterone treatment resulted in: (i) significant increase in the levels of acetylcholine and norepinephrine, (ii) significant increase in the cerebral cortex expression of Bcl-2 and (iii) significant decrease in the cerebral cortex level of MDA as well as a significant increase in the enzymatic (GSH) and non-enzymatic (SOD and CAT) antioxidants in cerebral cotex tissue in a rat model of vascular that is induced through BCCAO.
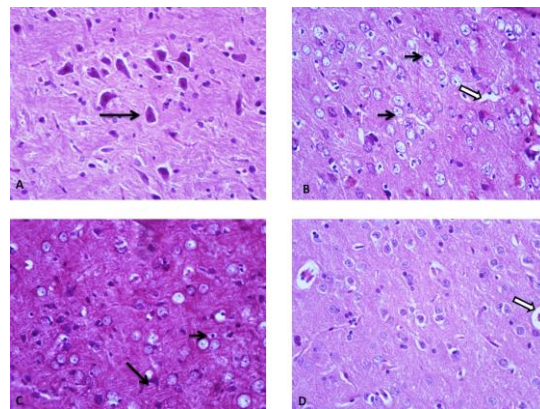
Fig. (3): Histopathological findings of the cerebral cortices of the control and experimental rats (H and E, original magnification X400)
(A) section from a control rat show intact neurons and well-preserved cell density
(B) section from the BCCAO rats shows neuronal loss with ischemic changes (cytoplasmic eosinophilia, pyknotic nuclei, pancellular necrosis, indicative of cerebral infarction and severe neuropilarmicrovacuolation).
(C) BCCAO +testosterone treated rats shows more or less intact neurons with intact chromatin.
(D) BCCAO + donepezil treated rats shows the

same ischemic changes but less than the BCCAO rats.

In the present study we had been using bilateral common carotid artery occlusion to produce a rat model of vascular dementia. Permanent, bilateral occlusion of the common carotid arteries (2VO) in rats has been documented as a procedure to study the effects of chronic cerebral hypoperfusion on cognitive dysfunction and neurodegenerative processes. Our results showed that BCCAO produced a significant decreased the central level of acetylcholine and norepinephrine in the cerebral cortex as compared with the control group. These data were in accord with previous studies that reported that cognition deficits and memory loss after BCCAO may be partly due to cholinergic dysfunction in the brain. Accordingly, the decrease of acetylcholine level in the striatum, cortex and hypothalamus has been observed up to four months post BCCAO. Interestingly, one month post BCCAO, a significant decline in acetylcholine was noted only in the striatal tissue.[21] The neurochemical data obtained in this study indicated that BCCAO leads to progressive and time-dependent neuronal damage and cellular necrosis as detected from histopathological examination (figure 4-B). On the other hand, testosterone treatment increased the cerebral cortex level of both acetylcholine and norepinephrine significantly as compared with BCCAO rats.

Also, testosterone treatment preserved the neuronal integrity and decreased the pyknosis and cellular necrosis. Testosterone and DHT have several important actions in the brain. Androgen actions are mediated in part via activation of androgen receptors (AR), which are localized in many brain areas including regions important for learning and memory such as hippocampus and amygdala. Beneficial actions of androgens in the brain include stimulation of neuronal differentiation, maintenance of neuronal morphology, and promotion of synaptic density For example, studies of hippocampus in male rats show a significant decrease in the density of spine synapses following gonadectomy (GDX), an effect reversed by replacement with either testosterone or DHT .In addition to androgen actions in neurons, testosterone has also been found to down-regulate astrogliosis.[22,23] Neuronal cell culture studies have revealed neuroprotective effects of androgens against serum deprivation and oxidative stress. Androgens such as testosterone and DHEA usually decrease with aging with development of adrenopause which consequently may predispose to Alzheimer disease (AD). Several studies now confirmed that circulating levels of testosterone are significantly lower in men with AD in comparison to age-matched, non-demented men, a relationship that appears strongest in men younger than 80

years of age. Previous studies revealed that androgen depletion results in increased levels of beta amyloid and hyperphosphorylated tau that led to subsequent neuronal death.[24] Androgen supplementation to hypogonadal men results in improved memory performance.[24]

BCCAO increased the oxidative stress in the cerebral cortex as compared to the control rats. Cerebral ischemia increased the cerebral cortex MDA as well as decreased the enzymatic (SOD and CAT) and non-enzymatic (GSH) antioxidants. Excessive ROS generation can induce the functional and structural damage of neuronal cells and may play an important role in the pathophysiology of cerebral ischemia.[25-27] The ischemic event begins with reduced blood flow to the areas supplied by the occluded arteries. The lack of oxygen, glucose, and other nutrients leads to a state of disturbed cellular homeostasis, culminating in cell death.[28] Testosterone treatment decreased the cerebral cortex MDA and significantly increased the antioxidant enzymes (SOD and CAT) and GSH as compared to BCCAO rats.

Interestingly, this study showed that cerebral ischemia decreased the expression of the anti-apoptotic Bcl-2 expression significantly as compared to control rats. Within a few minutes after ischemia, the neurons in ischemic core undergo irreversible injury. The cell death was traditionally thought to occur exclusively via necrosis; recent research indicated that apoptosis was the important cause of neuron death[29], especially the cells in penumbra.[30] Clinical evidence showed that apoptosis occurred in the penumbral region from 1 to 26 days after stroke.[31] Thus, suppressing apoptosis at early stage after stroke may be an important opportunity for salvage of neurons in penumbra, and then reduce the infarct volume and alleviate brain injury induced by ischemia.[32,33]

In this study, testosterone significantly increased the expression of Bcl-2 as compared to BCCAO rats in cerebral cortex tissue. Testosterone deficiency in males was associated with conditions that indicate CNS neuronal dysfunction such as depression, anxiety and memory loss.[34] Furthermore, replacement therapy significantly improved these symptoms. Also, androgens offered as much neuroprotection against growth factor deprivation mediated neuronal apoptosis of CNS differentiated human neurons as 17-bestradiol.[35]

In partial agreement with our data, testosterone and its metabolite 5-α dihydrotestosterone regulated the expression of Bcl-2 in human dermal papilla cells.[36] In disagreement with our data previous studies strongly suggested a proapoptotic effect of testosterone. Crisostomo et al.[37] performed a study examing testosterone and death signalling in rats. They found that

testosterone infusions for 5 min before myocardial ischemia enhanced Bcl2 expression and attenuated capase-3 expression.Estrada et al.[38] and Jia et al.[39] found similar effects of testosterone in the caspase-3 and Bcl-2 pathways, respectively, further suggesting a proapoptotic function of testosterone.In this study, a significant negative correlation betweenBcl-2 expression in the cerebral cortex and the MDA level in the homogenate of the cerebral cortex (r=-0.725, P<0.001). Previous studies linked the relation between Bcl-2 and oxidative stress. Bcl2 prevents the leakage of hydroperoxide through stabilization of the mitochondrial membrane potential[40]. The present research showed that testosterone might exert neuroprotective effects through increasing the expression of the cerebral cortex Bcl-2 and decreasing the oxidative stress.

CONCLUSION

Surgical bilateral common carotid artery in rats decreased the central level of acetylcholine and norepinephrine in the cerebral cortex as well as suppressed the expression of Bcl-2 in the rat cerebral cortex with increased oxidative stress. Treatment of the rat model of vascular dementia with testosterone increased the central level of acetylcholine and norepinephrine and upregulated the expression of Bcl-2 as well as increased the enzymatic and non-enzymatic antioxidants. Despite many of the experimental data suggest a beneficial effect for testosterone supplementation to produce protective effects in an animal model of vascular dementia and Alzheimer's disease, clinical trials in vascular dementia patients are still required to further validate many of the laboratory findings. The use of testosterone as a possible alternative treatment still requires further investigation, especially as part of a treatment protocol in clinical trials.

Conflict of interests: None declared.

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