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Low Doses of Tributyltin Chloride Induced Neurotoxicity in Male Rats

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ABSTRACT: The environmental contaminant tributyltin chloride (TBTC), which is still present in commercially-collected items, is majorly utilized and continuously dispersed. It is one of the chemicals which disrupts the endocrine system and is exceedingly harmful to a variety of organisms. In this work, male rats were given TBTC at doses between 10 and 2000 µg/kg B.W. to determine its neurotoxicity. Animals have been separated into 8 equal groups and given the next daily doses of TBTC through oral gavage for a period of 45 days: 10, 50, 100, 250, 500, 1000, and 2000 µg/kg B.W. The effects of TBTC administration. Along with disrupting brain neurotransmitters (acetylcholine, serotonin, and dopamine), TBTC elevated the indicators of the amyloid genetic pathway (amyloid beta protein 1-42 (AB1-42) and beta-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE-1), inflammation (nuclear factor kappa B), and apoptosis (Caspase-3 activity). Histopathological analysis revealed that TBT exposure altered the brain tissue architecture. The neurotoxicity caused by high TBTC doses was more significant compared to that caused by low doses, and the impact was dose dependent.

Keywords: *Tributyltin chloride; Neurotoxicity; Neurotransmitters; Molecular parameters; Histological changes* amyloid beta protein 1-42 (AB1-42) and beta-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE-1)

1. INTRODUCTION

Organotin compounds have a broader range of technological and industrial applications than any other metal's organic compounds. Catalysis or anticatalysts is used in many of these applications and are frequently found in polymer chemistry **(11)**. In addition, organotin compounds, like TBT are among the most dangerous pollutants released into the environment **(40).** Tributyltin and Triphenyltin compounds (chloride, oxide, acetate, etc.) were introduced to replace copper oxide, which had previously been used to decrease fouling growth **(11).**

Tributyltin poses a significant threat to ecosystems. Even at low concentrations, TBT compounds are highly toxic to various aquatic organisms. TBT is especially dangerous due to the fact that it builds up in both these organisms and the mammals and fish that eat them. The consumption of TBT-contaminated fish by humans can inhibit the defense mechanism. Touching with TBT-containing products can irritate the skin and eyes severely. According to studies, 2.4 billion gallons of water can become toxic to aquatic life after just one gallon of a 2 percent TBT solution is thrown down a drain. Hazardous compounds such as TBT continue to be present in the water dumped into the Bay because wastewater treatment plants are only intended to remove biological contaminants. TBT, because of its slow decomposition once released into environment, it might be a threat for up to ten years. **(2).**

In a three-generation investigation which has been carried out in a simulated setting, **(19)** found that TBT exposure produced endocrine, thyroid, and neurotoxicity disruption in zebrafish. TBT chloride was found to induce apoptosis in the hypothalamic neurons through triggering MKK4-JNK signalling pathway, according to **(3)**.

Nitric oxide (NO) and biomolecules like acetylcholinesterase (AChE) are essential for CNS functions like releasing neurotransmitters, regulating neuronal electrical activity, and changing synaptic plasticity **(38).**

Furthermore, tributyltin inhibits ATP synthase, which is in charge of ATP synthesis in the mitochondria. Inhibitors of this enzyme are thus potentially harmful to all life forms. TBT also prevents ATP hydrolysis by inhibiting ATPases **(36)**.

Tributyltin has the ability to alter chemical neurotransmitters and disrupt blood-brain barrier. Thus, the brain is being investigated as a possible target with regard to TBT toxicity **(24).** In addition, in TBT toxicity, apoptosis and oxidative stress have been thought to be inseparable phenomena **(22)**.

A high level of nitric oxide and increased acetylcholinesterase activity indicated neurotoxicity. In addition to histopathological changes, the dysregulated expression related to caspase-3, Bcl-2, and Bax indicated that TBT was causing apoptosis **(29)**. Tributyltin damages the bile duct and is harmful to the nervous system **(1)**. Tributyltin is more poisonous than dibutyltin **(35).** Even at very low doses, tributyltins are toxic to aquatic populations **(1)**. Tributyltin is a harmful buildup of marine creatures on solid exteriors absorbed in salt water like ship hulls/mechanical constituents. Tributyltin is simply captivated by creatures by digestion, and its cytotoxic impacts have been the main source of concern since its finding out in the 1970. The cardiovascular system is harmful to TBT exposure, according to current research (**27).** Tributyltin seems to exist as a membrane-active chemical whose effect is Organotin (OT) lipophilicity-dependent. TBT consequently penetrates cell membranes and damages endothelium as well as smooth muscle cells. Arterial dysfunction is brought on by TBT, primarily most probably due to the modifications to the vascular wall's morphology and endothelial dysfunction. **(27)**. TBT affects individuals by causing convulsions, excruciating pain, as well as mental health issues. As a result, TBT was already classified as a "very dangerous drug" by the European Commission **(6)**. DBTs as well as TBTs are neurotoxic and result in bile duct damage, The aim indicates that the DBT and TBT contamination is as highly diffuse in farmed as in free living fish and mussels on sale in retail markets in Naple's province even if the levels of the contamination are meanly quite low. **(1)**.

2. MATERIALS AND METHODS

2.1. *Tested compounds and doses*

Tributyltin chloride ($C_{12}H_{27}ClSn$) purchased from Sigma–Aldrich Chemical Company, St. Louis, MO, USA, in the form of clear liquid, and light yellow. Product No: T50202-5G, CAS- 1461-22-9, purity 96% and Molecular Weight: 325.51 g/mol. All reagents and chemicals were of analytical grade. The doses of tributyltin chloride were 10µg, 50µg, 100µg, 250µg, 500µg, 1000 µg, and 2000 µg/kg B.W. The doses were chosen according to **(24)**.

2.2. *Animals and experimental design*

Forty male Albino rats weighing 175 ±5 g (12 weeks old) were used in the present study. Animals were obtained from Faculty of Medicine, Alexandria University, Alexandria, Egypt. The research was carried out according to the Guide for the Care and Use of Laboratory animals (International Council for Laboratory Animal Science, ICLAS) and was approved by the local ethical guidelines of Institutional Animal Care & Use Committee (IACUC), Alexandria University, Egypt (AU14-211017-2-8) and all the methods were performed according to the guidelines and regulations of the same Committee. Rats were kept on basal diet and tap water is provided *ad libitum*. Animals were kept in normal atmospheric condition at a temperature of 25 ± 5 °C and 50-70% humidity were maintained throughout the experiments with a 12 h light/day cycle. After two weeks of acclimation, animals were randomized divided into 8 equal groups (5 rats per each group): group1 served as control and received vehicle dimethyl sulfoxide (DMSO) at dose ; groups 2, 3, 4, 5, 6, 7 and 8 were treated with TBTC at of 10µg, 50µg, 100µg, 250µg, 500µg, 1000µg, and 2000 µg/kg B.W., respectively. Animals were orally gavaged daily with respective doses for 45 consecutive days **(12).**

2.3. *Blood samples collection and tissue preparations*

Animals were sacrificed by the end of the experimental period using isoflurane 100 % (2 mg/Kg BW or equal 2% inhaled in dictator). Blood samples were collected by cardiac puncture and centrifuged for the separation of plasma (860 xg for 20 min) that was kept at -80 ◦C until analysis. Brain was immediately removed, washed using chilled saline solution (0.9 %), and removed the adhering fat and connective tissues. Brain was minced and homogenized (10 %, w/v), separately, in ice-cold sucrose buffer (0.25 M) in a Potter–Elvehjem type homogenizer. The homogenates were centrifuged at 10,000 xg for 20 min at 4 ◦C, to pellet the cell debris

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2 .4. *Quantitative analysis of brain inflammatory marker: nuclear factor kappa B*

Commercial ELISA kits (Chongqing Biospes, China) were used for determination of nuclear factor kappa B (NF-κB) in the brain tissue supernatants according to the manufacturer instructions.

2.5. *Apoptotic marker: Caspase-3 activity*

Caspase-3 colorimetric assay kit (Gen Script Inc., USA) was used. After cleavage from the labeled substrate DEVD-pNA, the chromophore pnitroanilide was quantified using a spectrophotometer at 400 or 405 nm.

2 . 6. 8-OH deoxyguanosine (8-OHdG)

Total DNA was isolated from brain tissues using DNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. 8-OH-dG was measured in DNA samples, using a commercial 8-OH-dG ELISA kit (Chongqing Biospes, China) following the manufacturer's protocol.

2 . 7. *Determination of nuclear factor kappa B (NF-κB)*

Commercial ELISA kits (Chongqing Biospes, China) were used for determination of nuclear factor kappa B (NF-κB) in the brain tissue supernatants according to the manufacturer instructions.

2.8. *Brain neurotransmitters*

Immunoassay kit (Chongqing Biospes Co., China) has been utilized for quantitative determination of the rat Ach, serotonin (5-hydroxytryptamine, 5-HT), in the cortical tissues. Rat dopamine (DA) levels in cortical tissues were determined quantitatively using an immunoassay kit from Chongqing Biospes Co. in China.

2.9. *Histological section preparation of brain*

Histopathological examination was carried out according to **(4)**. Brain were obtained from rats, and immediately fixed in 10% formalin, and then treated with a conventional grade of alcohol and xylol, embedded in paraffin and sectioned at 4-6 µm thickness. The sections were stained with Haematoxylin and Eosin (H&E) stains and photographed on the PC screen using a light microscope with a digital color camera attachment and dial indicator for studying the histopathological changes.

2.10. *Statistical analysis of the data*

Mean and standard error values were determined for all the parameters and the results were expressed as mean ± standard error. The data were analyzed using a one-way analysis of variance (ANOVA) followed by Duncan multiple comparison. $P<0.05$ was statically significant according to **(25)**.

3. RESULTS AND DISCUSSION

3.1. Neurotransmitters

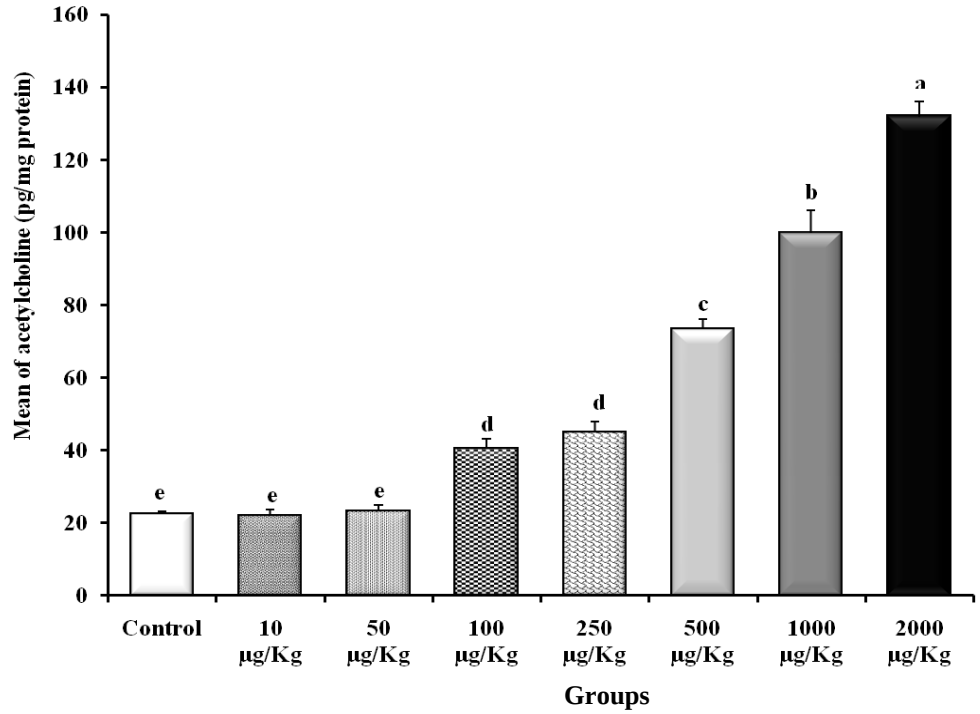
showed the changes in acetylcholine, serotonin and dopamine in brain of the male rats that have been supplemented with different doses of TBTC daily for 45 days. The results showed that the three neurotransmitters insignificantly changed in the groups that have been supplemented by 10 and 50 µg /kg bw TBTC, while significantly increased in the groups that have been supplemented by 100, 250, 500, 1000, and 2000 µg /kg of TBTC when compared with control groups **Figure(1)**.

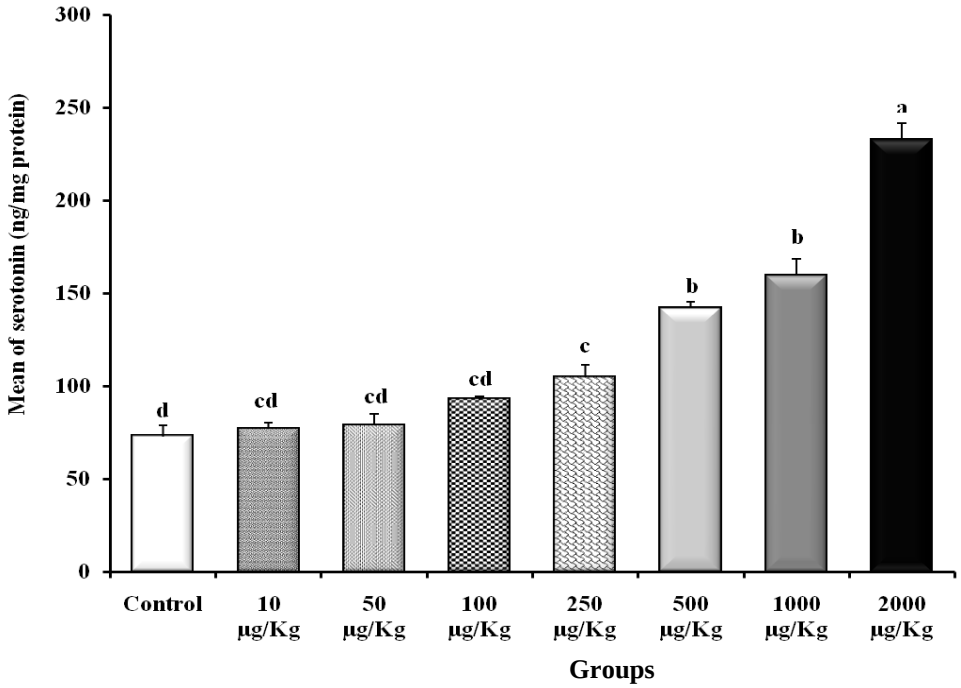
Neurotransmitters are necessary for nervous system development and behavior regulation. The brain could be one of TBT's possible target organs. A neurotoxicological study has shown that levels of dopamine, norephinephrine and serotonin in the rat brain decreased in a TBT dose-dependent manner **(5)**. This has been in accordance with the present data that had shown a significant decrease in acetylcholine, serotonin and dopamine in the brain of male rats that have been supplemented with different doses of TBT.

demonstrated that many molecular components, including AChE and nitric oxide (NO), are involved in process of neurotransmission and neural function regulation **(32)**. Controlling the actions of acetylcholine and AChE play an important part in cholinergic synapses' synaptic transmission. AChE dysfunction is also linked to several neuropsychiatric disorders, according to the research of **(38).**

Dopamine and serotonin (5-HT) are neurotransmitters that regulate many CNS functions as well as synapses(**42)**. extrinsic endocrine disruptors like TBT could interact through any neuro communication **(12**) , altering neurotransmitter transport and leading to changes in behavior. reported that fish treated with TBT exhibited elevated concentrations of dopaminergic (DA) as well as serotonin (5-HT) **(41)**. However, according to **(8)**, TBT treatment may harm neurons by increasing oxidative stress within the brain**.** have also demonstrated that TBT neurotoxicity results from modulating levels of neuro-transmitters in different brain zones of the mice **(34)**. also revealed that TBT inhibited the synthesis of dopamine by reducing the activity and expression of tyrosine hydroxylase**(17)** ; the rate-limiting step in dopamine biosynthesis, in PC12 cells. The striatum, an area of the brain that is rich in dopamine is where TBT produces much more damaging effects **(17)**. According to **(9)**, TBT might even inhibit several parameters of cholinergic activity within the cortex. Also, TBT administration may lower serotonin levels inside the striatum/cerebellum **(34)**.

According to TBT administration lowered certain neurotransmitter concentrations within different brain regions**(18)** , except for the cerebellum. The main neurotransmitters implicated in neurobiology for depression, serotonin, noradrenaline (norepinephrine), glutamate, as well as dopamine, were demonstrated to be modulated by ROS, according to research by . Severe depression has been associated with low concentrations of various endogenous antioxidant substances and enzymes along with a decline in the overall antioxidant system (**30)**.





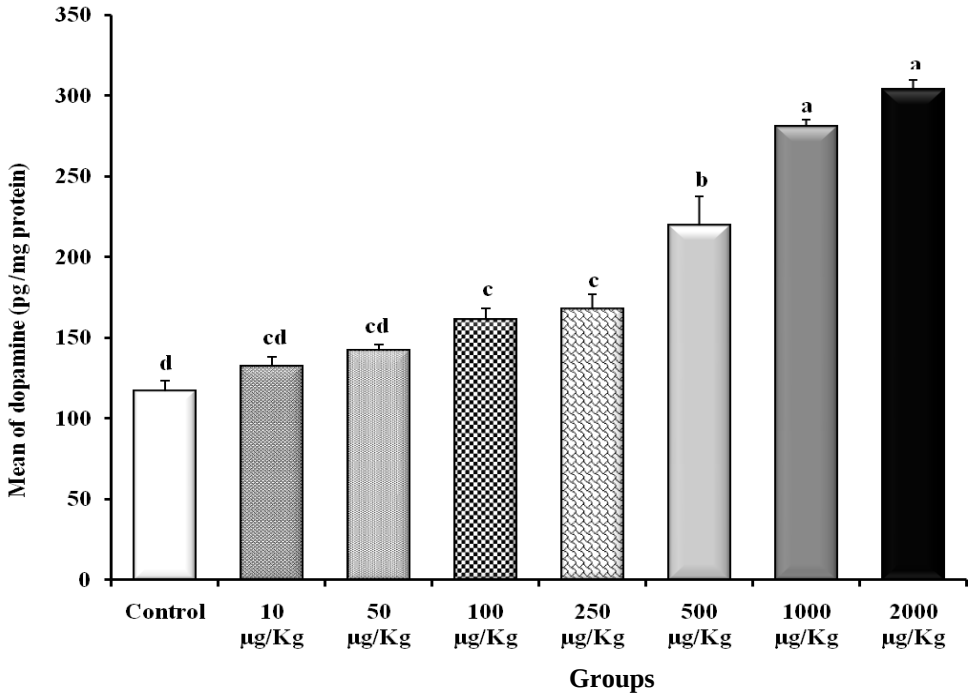


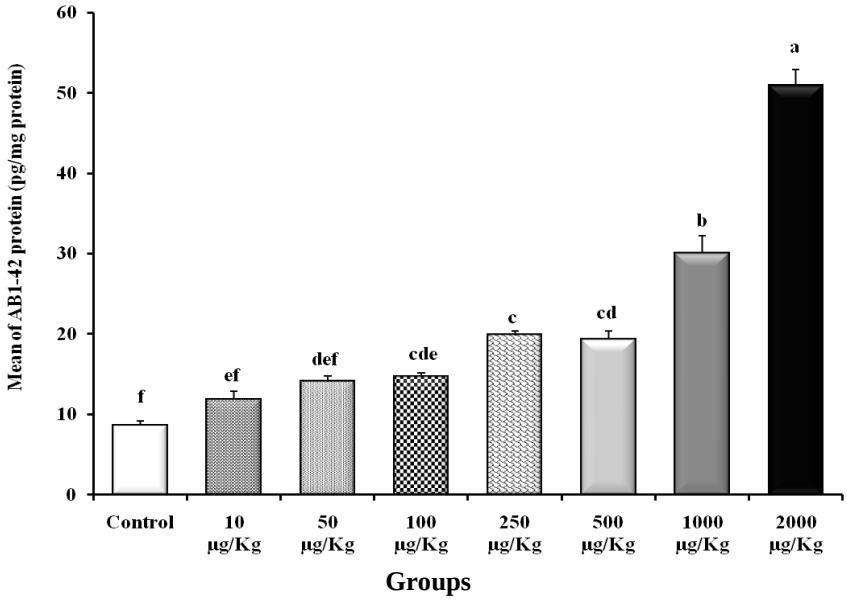
Figure 1: Changes in cortical neurotransmitters; acetylcholine, serotonin and dopamine in the brain of male rats administered tributyltin chloride (TBTC) For 45 days

Value expressed as means ± S. E; n = 5 rats each group. Superscript letters (a, b, c, d, e) are significantly different at $p < 0.05$. Mean with letter a is the highest one, followed by another letter, then e is the lowest one .

3.2. *Changes in the amyloid genic pathway*

showed the changes in the amyloid beta protein 1-42 (AB1-42) and beta-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE-1), pathway in the cerebral cortex of the brain of male rats that have been supplemented with different doses of TBTC daily for 45 days. There was no discernible change in AB1-42 protein or BACE1 in the rats that have been treated by 10 or 50 g/Kg TBTC compared to the controls. AB1-42 protein and BACE1 levels both increased significantly relative to the control group as TBTC dose has been increased in the rats **Figure (2**) .

The increased expression of BACE1 in the brain cortex, which is correlated with significantly higher levels of A1-42, suggests that TBT treatment activates the amyloid genic pathway. The BACE1 gene encodes the -secretase enzyme that catalyses the -secretase-site cleavage of (APP) to generate amyloid beta-protein 1-42 (A1-42) **(37).** One possible predictor of Alzheimer's disease is an amyloid genic pathway which has been activated(AD Alzheimer's Disease).



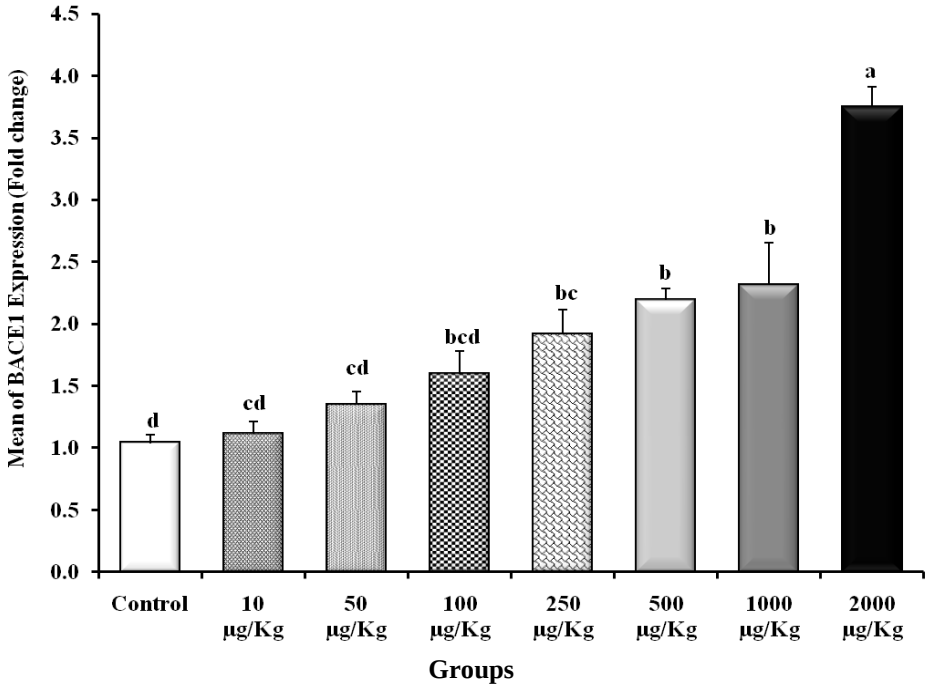


Figure 2: Changes in the components of amyloidogenic pathway; amyloid beta 1-42 protein and BACE1 gene expression in the brain of male rats that have been supplemented with tributyltin chloride (TBTC) For 45 days

Value expressed as means ± S. E; n = 5 rats each group Superscript letters (a, b, c, d, e) are significantly different at $p < 0.05$. Mean with letter a is the highest one, followed by another letter, then e is the lowest one

3.3. *The inflammatory and apoptotic markers*

showed the changes in the Nuclear Factor Kappa B (NF-κB), and Caspase3 in the brain of male rats that have been supplemented with different doses of TBTC daily for 45 days **Figure (3)**. The results showed that NF-κB insignificantly changed in the groups that have been supplemented by 10 and 50 µg /kg bw TBTC, and significantly increased in groups that have been supplemented with 100, 250, 500,1000, and 2000 µg/kg of TBTC when compared with the control group. Starting from the dosage of 250 µg /kg bw TBTC and along with elevating the dose the activity of caspase-3 had shown a significant increase in comparison to control groups. On the other hand, NRF2 had been significantly increased in the groups that have been supplemented by 10 and 50 µg/kg of TBTC. The NRF2 had shown a significant dose-dependent decrease in groups that have been supplemented with 50,100,250,500,1000 and 2000 µg /kg of TBTC compared with the control group. 8-OH-dG insignificantly changed in groups that have been supplemented by 10, 50, and 100 µg/kg of TBTC and significantly increased in the groups that have been supplemented by 250, 500, 1000 and 2000 µg/kg of TBTC compared with the control group.

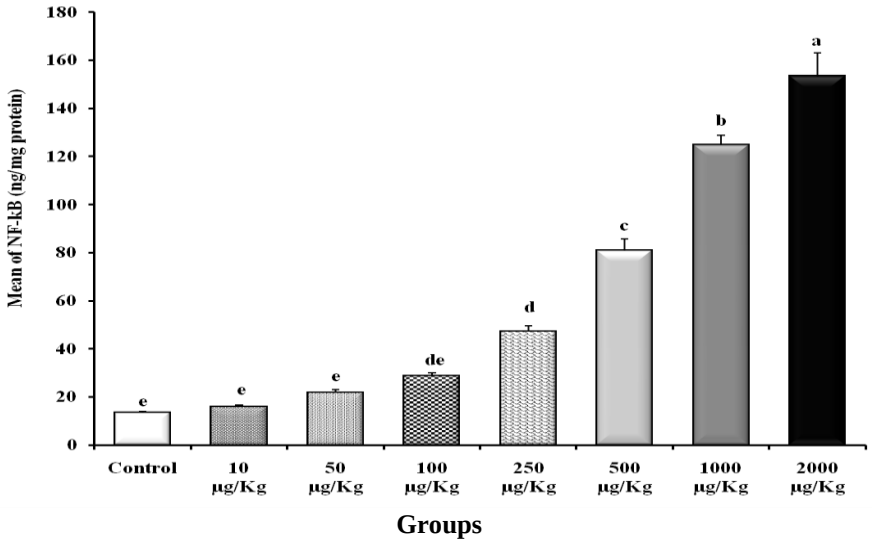
According to **(23)**, a single acute dosage of TBTC alters metal homeostasis, damages the BBB over the long term, promotes inflammation and oxidative stress, and encourages caspase-mediated cell death through apoptosis in the p38 signaling pathway. Elevated concentrations of NF-kB signal inflammatory pathways seriously compromise the integrity of the BBB. An increase in NF-kappaB content in the cerebral cortex was associated with TBTC's upregulation of GFAP expression, a mediator of the inflammatory response. Several neurodegenerative illnesses can be caused by the oxidative stress and BBB distortion triggered by these responses if they persist for too long**.**

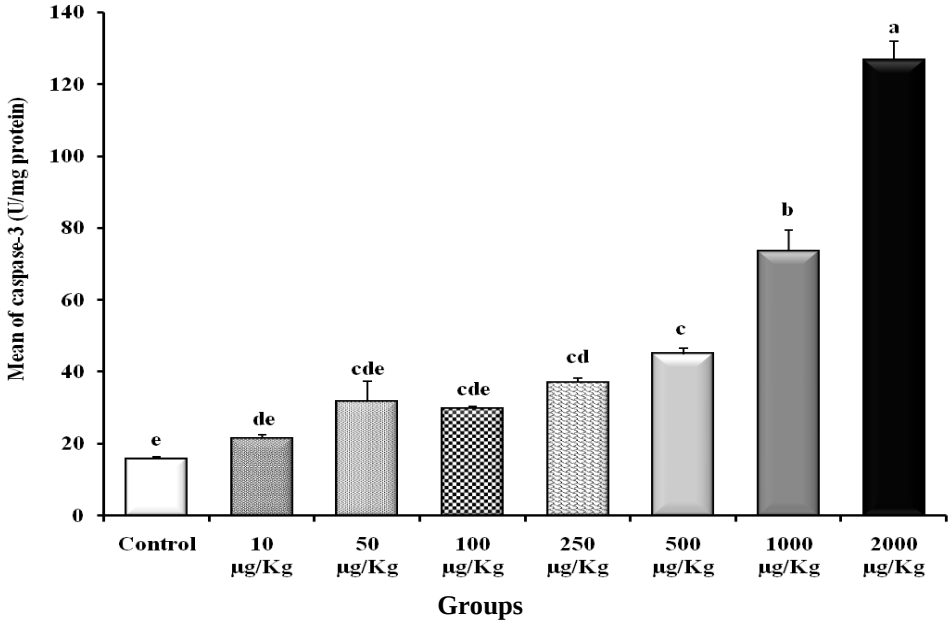
A group of transcription factor proteins known as NF-κB orchestrates several genes which regulate immune function. The inhibiting protein called inhibitor of B (IκB) coexists inside the cytoplasm with NF-B subunit proteins p65 as well as p50 **(20)**. The NF-κB responses to TBT stimulation were assessed in rats, overall transcription of the p50, as well as p65 subunit proteins, increased whereas the synthesis of the (IκB) subunit protein reduced concerning NF-κB. Elevated NF-κB protein expression (p-65) has been linked to the neuronal inflammations in the rat brain after the exposure to the TBT (10–30mg/kg) for 7 days, according to (**23)**.

Caspase-3 stimulation with mitochondrial membrane depolarization leads to cell death via apoptosis. Caspase-3 may be the last effector, but it also triggers the cleavage and activation of caspase-8, upstream initiator, of apoptotic neuronal cell death**. (19)** also investigated the neurotoxicity generated by TBT treatment and found an upregulation of caspase-3 activation in the zebrafish brain following TBT induction.

In the hippocampus slices, **(15)** discovered that nucleosomal DNA fragmentation happened after TBT-induced ROS generation. In general, ROS triggers several intracellular processes that all result in cell damage, such as kinase signaling, mitochondrial malfunction, and/or caspase activation, (**7)**. Furthermore, researchers noted significant oxidative stress

decreased permeability of mitochondrial membrane that was accompanied by nucleosomal DNA breakage induced by endonuclease G, which had been transferred from either the mitochondria towards the nuclei.
During apoptotic procedures, active caspases break the inhibitor, causing caspase-activated DNA fragmentation **(28)**. Upon DBT stimulation, DNA fragmentation was found to increase in the hippocampus and the cortex **(16)**.
Tributyltin has been shown by **(23)** to directly suppress the expression of the NRF2-antioxidant system in rats, a primary molecular target in the fight against oxidative stress. Transcription of the antioxidants is governed by NRF2, the master transcription factor. When cells are exposed to free radicals, NRF2 moves into nucleus, where it binds to the antioxidant response elements (AREs), triggers the production of phase II detoxifying enzymes and antioxidant enzymes via the ARE, and increases cell resistance to free radicals and nucleophilic chemicals **(33)**.
Enhanced protein agglomeration may result from oxidative protein harm, while cellular dysfunction may result from oxidative stress to lipids **(14).**
Oxidative stress has been considered as one of the key contributors to onset of brain injury. The oxidative damage not only cause functional and structural changes, but it could also have an impact on cell mortality through apoptosis or necrosis **(10)**. Results of showed a dramatic rise in MDA levels in the intestines of juvenile common carp exposed to TBT at 7.5g/L for 60 days (21). Also, reported that the lipid peroxidation marker MDA significantly increased in brains of juvenile Japanese medaka following the administration of high dose of TBT**(31)**. In rat hippocampus slices, TBT exposure led to the formation of lipid peroxidation, ROS, as well as eventual cell death **(13)**. TBT-induced, cell damage may be mediated as well through the hydrogen peroxide as well as radical entities, including the hydroxyl radical according to **(25)**





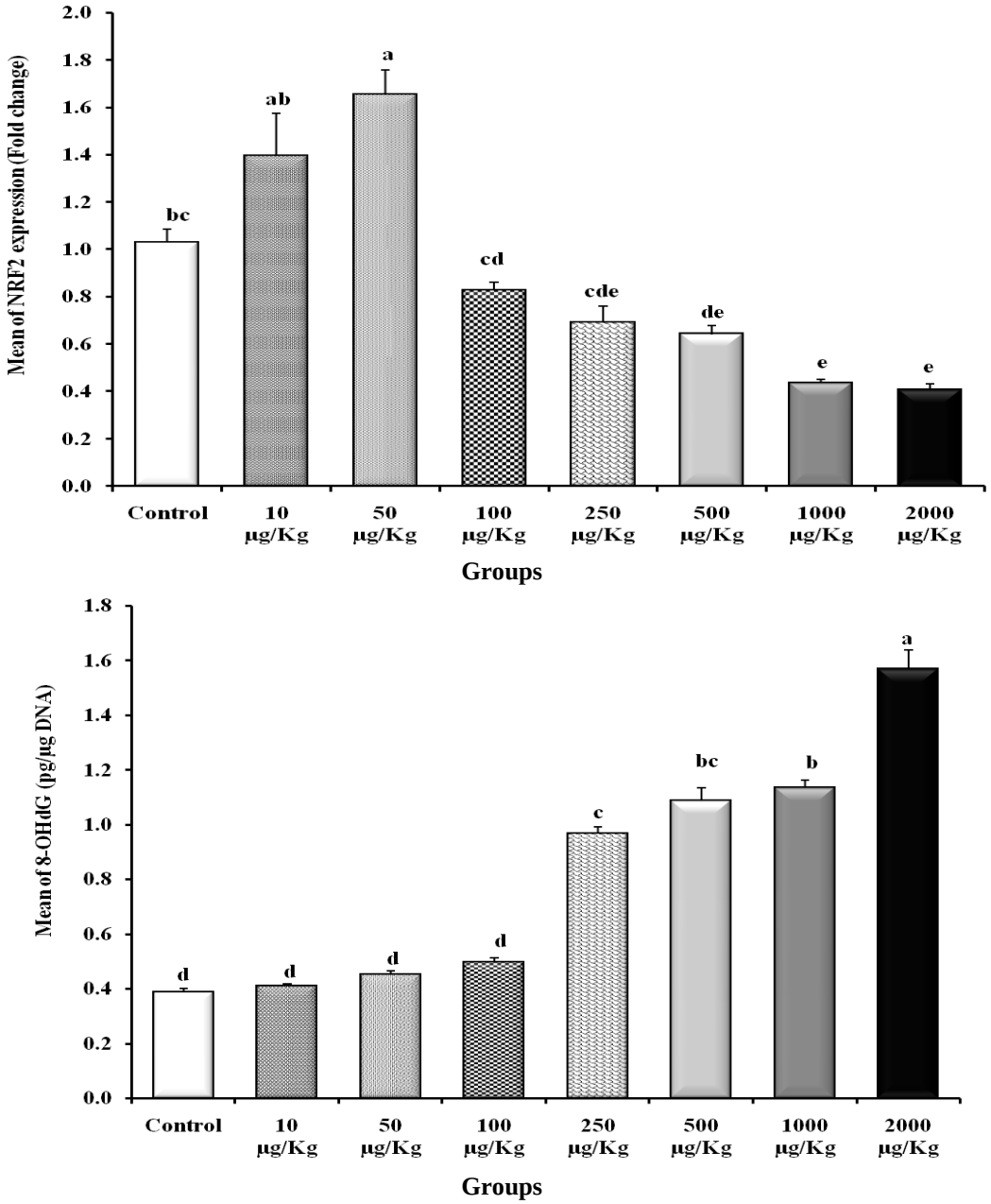


Figure 3: Changes in the inflammatory marker; NF-kB , nuclear factor erythroid 2-related factor 2 , apoptotic marker; caspase-3 , and 8-OHdG activity in the brain of male rats that have been supplemented with tributyltin chloride (TBTC) For 45 days

Value expressed as means ± S. E; n = 5 rats each group. Superscript letters (a, b, c, d, e) are significantly different at $p < 0.05$. Mean with letter a is the highest one, followed by another letter, then e is the lowest on.

3.4: *histopathological Changes of brain tissues*

Microscopic examination of H&E stained photo micrograph sections of rat brain assessing the neurotoxicity of different doses (10, 50, 100, 250, 500, 1000, and 2000 µg/kg bw) of tributyltin chloride (TBTC); showed, in control male rats brain normal architecture of brain tissue, normal nerve fibers, normal appearance of the pigmented neurons of the substantianigra, and normal oligodendrocytes **(Figure 4)** However, brain tissue of rats that have been treated by TBT (10µg/Kg body weight) revealing also, normal architecture of brain tissue, with normal appearance of the pigmented neurons of the substantianigra and normal oligodendrocytes **(Figure 5)**.

Meanwhile, brain tissue of male rats that have been treated by TBT (50µg\Kg body weight) illustrating, normal architecture of brain tissue, normal of nerve fibers, normal oligodendrocytes with mild chromatolysis on nuclear material **(Figure 6)** On other hand, brain section of male rats that have been treated by TBT (100µg/Kg body weight) demonstrated,

abnormalities in brain tissue architecture, where Apparant mild vaculation, mild spongosis, with mild degeneration in cells and mild lesions of neuronal cells and damage accompanied with pyknotic glial cell gliosis **(Figure 7)** whereas, brain tissue of male rats that have been treated by TBT (250µg/Kg body weight) demonstrated, moderate Degeneration in brain tissue architecture and disarrays in cell distribution, accompanied by mild vaculations within the tissue cells, moderate spongosis, mild Degenerationin of brain cells, accompanied by mild lesions and neuronal damage with gliosis mild presence of pyknotic glial cells **(Figure 8).**

Moreover, brain tissue of male rats that have been treated by TBT (500µg/Kg body weight) demonstrated, disarrays in cell distribution, moderate lesions of neuronal cells, mild presence of inflammatory cells, and mild neuronal damage with apparent gliosis, moderate vaculations and moderate spongosis, mild degenerationin in brain cells and moderate presence of pyknotic glial cells **(Figure 9) .**

On other hand, brain tissue of male rats that have been treated by TBT (1000µg/Kg body weight) showed, severe degeneration of brain tissues architecture and cells, with apparent disarrays in brain cell distribution, severe presence of vaculations with moderate spongosis, mild lesions of damage neuronal cells are seen, with moderate presence of gliosis, accompanied by severe invasion of inflammatory cells, and mild rate of neuron necrosise, accompanied by moderate presence of pyknotic glial cells **(Figure 10)** Moreover, brain tissue of male rats that have been treated by TBT (2000µg/Kg body weight) illustrated, severe degeneration in brain tissues architecture and severe disarrays in brain cell distribution with apparent irregular cell morphology and distribution, severe congestion of cerebral blood vessel and severe focal Hemorrhage from blood vessels was well noticed, moderate neuron necrosis and lesions of neuronal fibers, severe presence of vaculations with severe spongosis within brain tissue **(Figure 11)**.

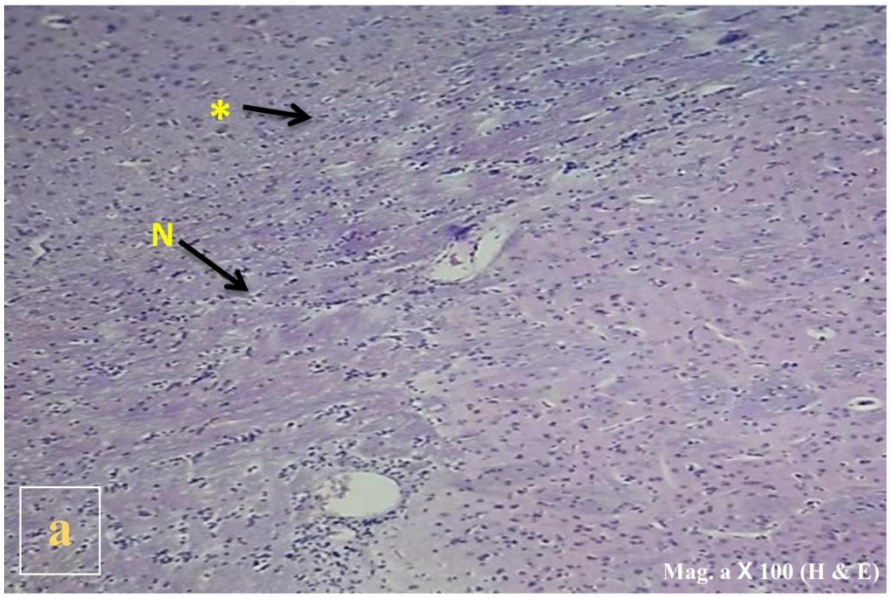


Figure (4): **Representative hematoxylin and eosin-stained brain sample at 100x of control (C) group showed normal of nerve fiber (N), normal appearance of the pigmented neurons of the substantianigra (*) Normal oligodendrocytes (CELL), the cells must be power of magnification figure in 400x.**

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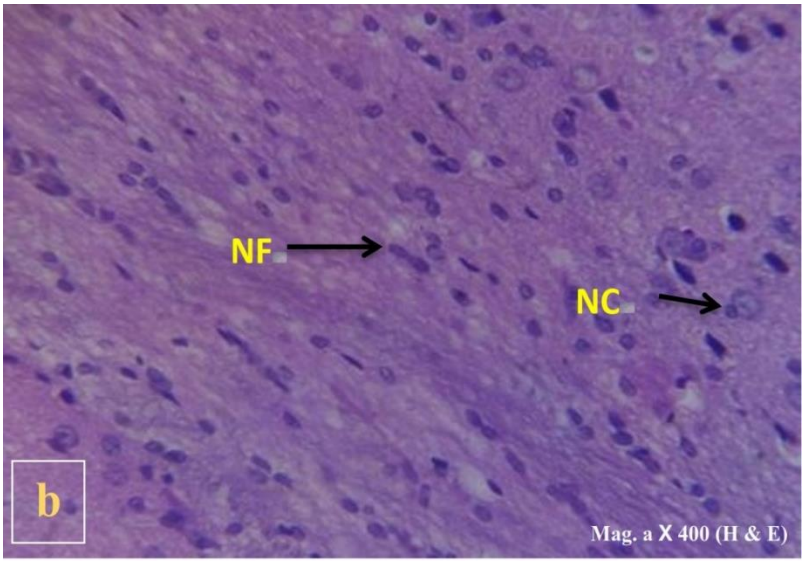

Figure (5): **Representative hematoxylin and eosin-stained brain sample at 100x of group treated with dose10µg TBT showed normal of nerve fiber (NF), normal appearance of the pigmented neurons of the Substantianigra (*) Normal oligodendrocytes (CELL), in 400x.**

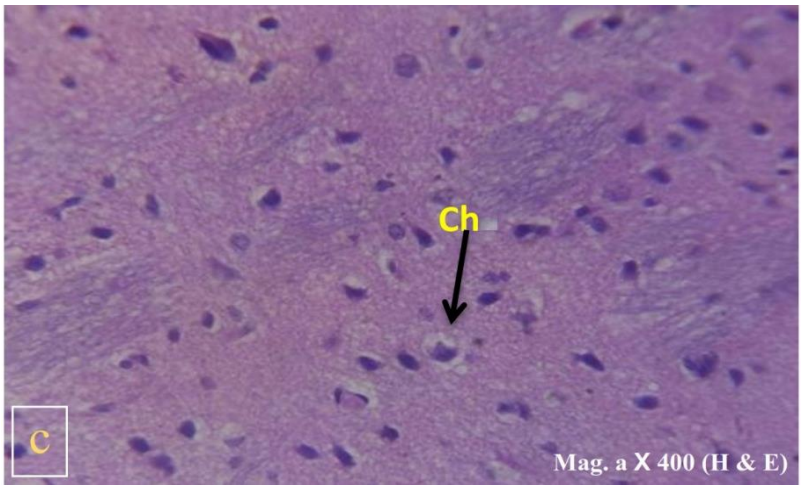

Figure (6): **Representative hematoxylin and eosin-stained brain sample at 100x of group treated with dose 50µg TBT showed normal of nerve fiber (NF), slight chromatolysis on nuclear material Normal oligodendrocytes (CELL), in 400x.**

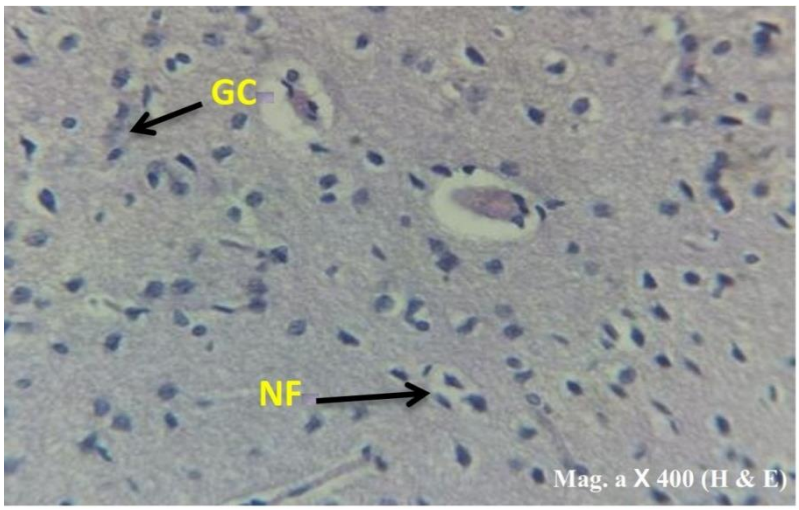

Figure (7): **Representative hematoxylin and eosin-stained brain sample at 100x of group treated with dose 100µg TBT showed vaculation and spongosis, degeneration in cells mild lesions of neuronal damage with gliosis (pyknotic glial cell) (red arrow), in 400x.**

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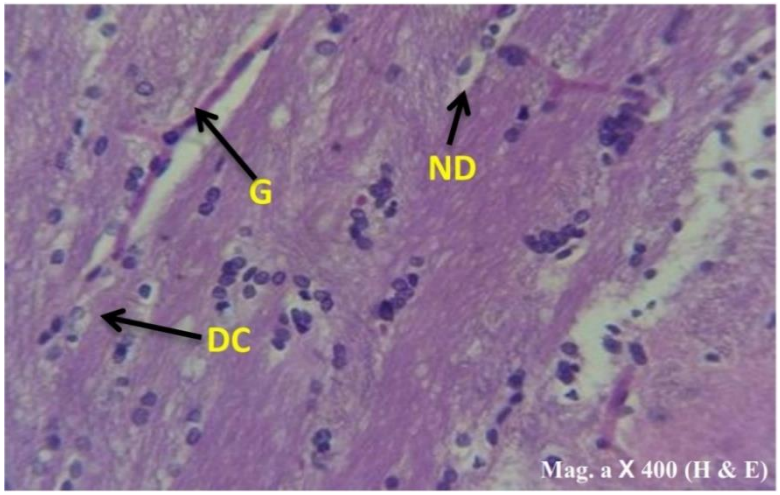

Figure (8): **Representative hematoxylin and eosin-stained brain sample at 100x of group treated with dose 250µg TBT showed vaculation and spongosis, degenerationin cells mild lesions of neuronal damage with gliosis (pyknotic glial cell) Disarrays in cell distribution, in 400x.**

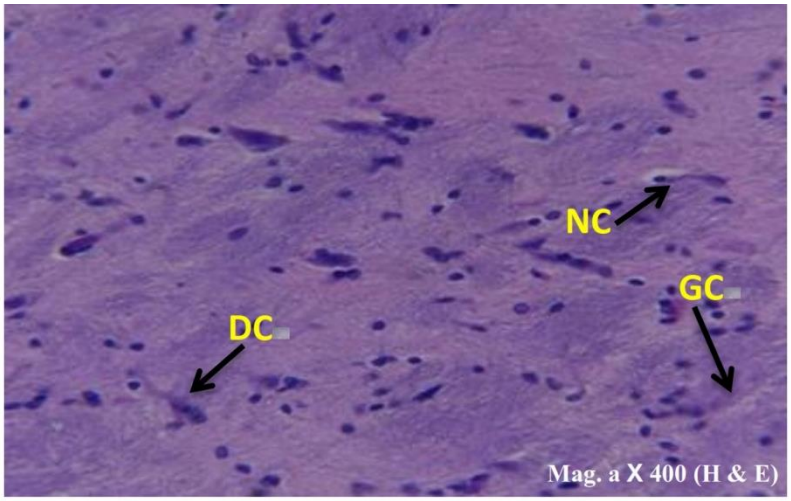

Figure (9): **Representative hematoxylin and eosin-stained brain sample at 100x of group treated with dose 500µg TBT showed vaculation and spongosis, degenerationin cells (DC) mild lesions of neuronal damage with gliosis (pyknotic glial cell, GC), inflammatory cells Disarrays in cell distribution, in 400x.**

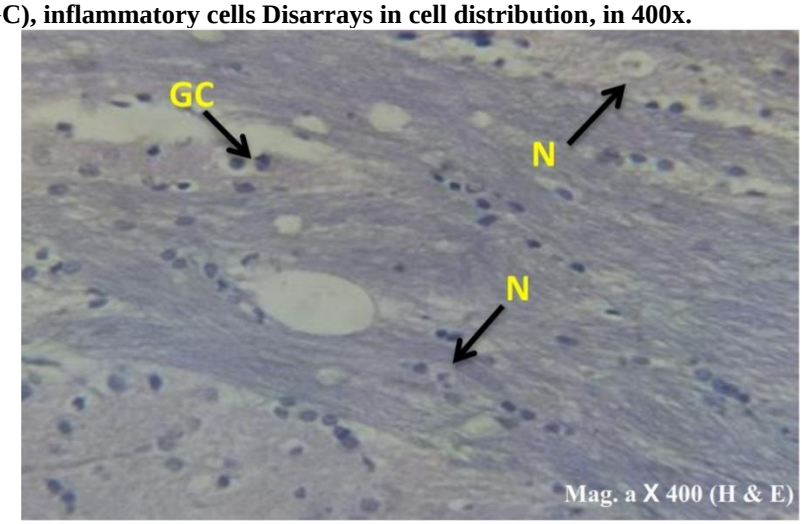

Figure (10): **Representative hematoxylin and eosin-stained brain sample at 100x of group treated with dose 1000µg TBT showed vaculation and spongosis, degenerationin cells mild lesions of neuronal (N) damage with gliosis (pyknotic glial cell, GC), inflammatory cells. early neuron necrosis Disarrays, in 400x.**

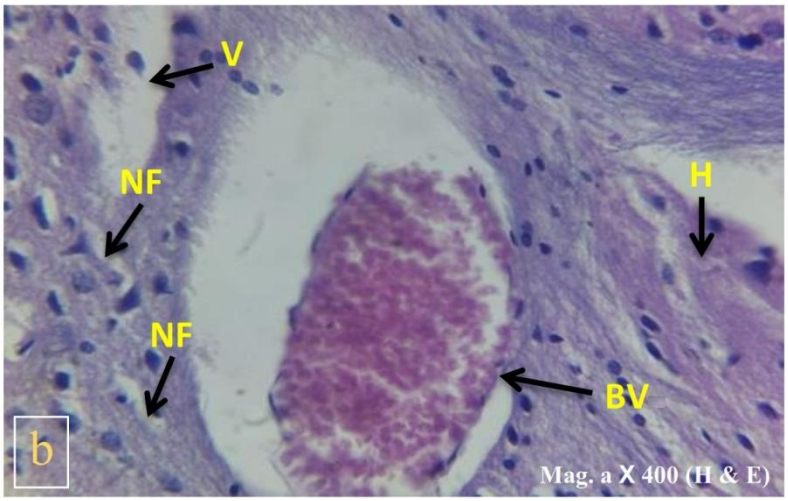

Figure (11): **Representative hematoxylin and eosin-stained brain sample at 100x of group treated with dose 2000µg TBT showed congestion of cerebral blood vessel (BV) and focal haemorrhage (H), irregular cell morphology and distribution, early neuron necrosis, lesions of neuronal damage with gliosis (pyknotic glial cell), Increased Vaculation and spongosis, High degenerationin cells, in 400x .**

4. CONCLUSION

From the results of this study, it can be concluded that TBTC induced changes in molecular parameters and histopathology in brain tissue. Des-regulation of the brain neurotransmitters, induction of neuro-inflammation and neuronal apoptosis.

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