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RESEARCH PAPER

Study on the protective effect of green tea against bisphenol A- induced amyloid aggregation in the brain of male albino rats.

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A B S T R A C T:

Bisphenol A (BPA) has been used in the plastic industry and widely distributed in the environment. Green tea extract has been known for its antioxidant activity. This study was designed to investigate the protective efficacy of green tea against brain amyloid aggregation which induced by BPA. Twenty male albino rats were exposed to BPA (500 mg/kg); green tea ethanolic extracts in two dose levels 150 and 300 mg/kg respectively plus BPA. After 60 days, malondialdehyde (MDA), superoxide dismutase (SOD) in the brain were measured besides body and brain weights. BPA exposed groups showed a significant reduction ($P<0.05$) in the final body weight, with a non-significant reduction in absolute brain weight. BPA was also increased oxidative stress in the brain of the treated rats besides histological changes with amyloid deposition in the brain detected by congo red staining technique. Treatment with green tea ethanol extracts were protected animal brains against adverse effects caused by BPA and reduced oxidative stress in a dose-dependent manner. that may be related to enriched ingredients that have been found in the extract.

KEY WORDS: BPA, brain, green tea extract, oxidative stress, MDA, SOD, amyloid
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1.INTRODUCTION

Bisphenol A (BPA) is one of the widely spread Contaminants that used in the manufacturing of polycarbonate plastic (Hernandez-Rodriguez et al., 2007). It brought extensive attention regarding the different ways of human exposure; orally by leaching from plastic containers or plastic lining of cans containing food, or by inhalation through incorporation by dust (Hugh S. Taylor, 2008).

Studies revealed that BPA causes adverse effects on the brain, reproductive system (Lang et al., 2008), and liver (Nakagawa and Tayama, 2000). BPA also brought considerable attention due to its estrogenic activity (Zuo and Zhu, 2014).

Amyloid beta(Aβ), toxic protein accumulation in the central nervous system (CNS), has been correlated with certain neurodegenerative diseases like Alzheimer's disease (Prasansuklab and Tencomnao, 2013). A previous study indicated that BPA has a direct connection to the accumulation of amyloid polypeptide in the human pancreatic islets which lead to the death of the insulin- producing cell and finally leads subsequently to type 2 Diabetes Mellitus (Gong et al., 2013). Later BPA was

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found to disturb insulin signaling pathways and increases amyloid precursor protein in the brain cortex of mice offspring (Fang et al., 2016a). BPA considered as one of the risk factors contaminates that accelerate the appearance of Alzheimer's dementia in addition to other factors like fluoride and aluminum (Mendelson, 2009).

Green tea is a favorite beverage which first discovered and spread from China to the world, green tea is a less processed type of tea (*Camellia sinensis*) without fermentation, this type of tea contain a pharmaceutical valued ingredients contents like flavonoids, Catechins, tannin, amino acids, and vitamin C (Katiyar and Elmets, 2001, Elżbieta SIKORA, 2011). Catechins belong to the phenolic acid components of green tea, which composed of eight types and epigallocatechin- 3-gallate (EGCG) represents the most abundant catechin in the green tea (Lu and Chen, 2008).

Recently green tea has been considered as a new therapeutic approach in the treatment of several diseases like Parkinson disease as it contains a very important phenols Epigallocatechin-3-gallate (Jurado-Coronel et al., 2016). It also used in the treatment of cancer, high blood pressure, and inflammation (Riegsecker et al., 2013), With the treatment of dyslipidemia in the blood of overweight and obese people (Yuan et al., 2018).

This study is an attempt to illustrate the role of BPA in the deposition of amyloid protein in the brain. which may be in charge of accelerate aging processes in the brain and also to investigate the ameliorative effect of green tea extract on the adverse effects of BPA as green tea was known to contain unique ingredients of medical importance.

2.MATERIAL AND METHODS

Green tea (China) ethanol extract was prepared according to the way that was used by Hernandez-Perez et al., (1994). 100 g of dried plant powder was mixed with 1000 ml of ethanol (95%). Put in a horizontal shaker for 24 hours, then was separated by filter paper, and then was centrifuged for 3000 g. Crude extract was obtained by filtration followed by evaporation of the solvent in a rotatory evaporator in 40ºC under low pressure. The extraction was kept in 8ºC till usage and re-suspended with distilled water to prepare the stock solution.

This study was carried out on 20 *Wistar* male albino rats weighing between (180-200) g obtained from the college of veterinary medicine in Baghdad/Iraq. All animals were housed in polypropylene cages with mesh wire tops in a well-ventilated room and provided with balanced ration and clean water *ad libitum*, with 12:12 light /dark photoperiod at 22±4ºC. Rats were divided into four equal groups (5 animals each). First group was treated with500mg/kg BPA (Solar bio company, Beijing, China) melted in corn oil; the second group treated with a mixture of BPA (500 mg/kg) plus green tea extract 150 mg/kg; third group was treated with BPA plus green tea extract 300mg/kg; the fourth group represents the control group treated with corn oil only. Animal dosing was orally for 60 days. Treatment with green tea extract was conducted after one hour of BPA dosage.

At the end of the experiment, animals were fasted overnight and weighed then anesthetized by ketamine and xylazine. During dissection, brains of the treated rats were removed, and half of the brain was washed with cold saline. Dried and then weighed and kept in -80ºC until the preparation of tissue homogenate to measure malondialdehyde (MDA), and superoxide dismutase (SOD).

For the preparation of Brain tissue homogenate, 0.1 gm. of the brain was mixed with 1 ml of extraction solution accompanied by the commercial kits (solar bio co., Beijing, China) used for the measurement of SOD and MDA. Then the tissue was homogenized or fully ground by Dounce homogenizer in ice by 8000 rpm 4ºC and centrifuged for 10 min. The supernatant was used for the test.

Another part of the brain was fixed in 10% neutral buffered formalin. Paraffinized brain tissue blocks were processed and cut by a microtome at 5 µm thickness, then deparaffinized, and counterstained by hematoxylin and eosin (H&E) to study histological changes.

Modified Higman's Congo red was used for the detection of amyloid plaques in the brain tissue. (BANCROFT et al., 1990). Histological sections 5µm thicknesses were deparaffinized by xylene then rehydrated by ethanol, washed by distilled water (D.W.) for 1 minute. Stained with Congo red solution for 20 minutes, differentiate quickly to alkaline alcohol for 5 to 10 dips, then rinsed for 1 minute in tap water, counterstained

with Gill hematoxylin for 30 seconds. Washed for 2 minutes by running water then dehydrated by ascending serial of alcohol (95% twice,100%) each change for 3 minutes, cleared with xylene (twice each change for 3 minutes) then mounted with Canada balsam and covered. Finally, slides were examined under a polarized light microscope for the detection of amyloid protein.

Statistical analyses were conducted using GraphPad Prism software version 6.0 (GraphPad, San Diego, CA). Data are presented as means with their standard error of the mean (mean ± SEM). Normality and homogeneity of the data were confirmed before ANOVA, and differences among the experimental groups were assessed by one-way ANOVA followed by Turkey's test. A probability level of $P<0.05$ was, considered as statistically significant.

3.RESULTS

Oral administration of BPA induced significant weight reduction ($P<0.05$) in the final body weight in comparison with the control group and a non-significant decrease in brain weight (Table 1).

Green tea extract at a level dose of 150 and 300 mg/kg plus BPA were caused a gradient non-significant decrease in final body weight and brain weight when compared with BPA or control group. Although the decrease was not statistically significant, the decrease in body weight induced by BPA was slightly improved by green tea in both dose levels, but still lower than the average value when compared with the control group.

The obtained results (Table1) revealed that brain MDA levels were significantly ($P<0.05$) increased in the BPA treated group when compared to the control group. Oral administration of green tea extract shows an obvious decreasing in the MDA brain level in a dose- dependent manner. The best lowering effect was seen in 300 mg/kg of green tea extract.

In contrast, BPA administration was significantly decreased SOD activities in rat brains when compared with the control group. The best results were seen with both doses 150 and 300 mg of green tea plus BPA. This increment was statistically significant ($P<0.05$) when compared with BPA group, while it was not significant when compared with the control group (table 1).

Histologically, BPA treatment induced several changes in the brain tissue (fig.1). There was congestion in the blood vessels with shrinkage and clot formation inside the blood vessels, hyaline necrosis in the cortex layer of the brain with gliosis (inflammation), pyknosis and disarrangement of Purkinje cells in the cerebellum. In green tea dose levels 150 and 300 mg/kg there was gradient improvement in the brain tissue depending on the dose used (fig. 2).

Congo red stained section under light microscope revealed the presence of pink and red plaques in BPA, which represents amyloid deposits. The plaques were seen in both cerebrum and cerebellum of the brain. While in green tea treated groups in both dose level 150 mg/kg plus BPA, there was a noticeable reduction in amyloid plaques which completely removed in the higher dose of green tea (300) mg/kg (fig. 3).

Table 1. Effect of different treatments on final body weight, absolute brain weight, SOD and MDA levels in the brain homogenate.

Treatment	Initial body weight(g)	Final body weight(g)	absolute brain weight(g)	SOD (U/g)	MDA(nmol/L)
Control	221.8±15.79	275±7.36	1.852±0.04	649±59.29	36.38± 1.24
BPA	174.4±15.34	192±26.01[a]	1.484±0.11	383±30.79[a]	47.55±1.10[a]
BPA+green 150 mg	218.2±13.44	239±13.77	1.794±0.10	678±13.47[b]	42.19±0.42[bc]
BPA+green 300 mg	197.0±9.935	251±21.52	1.782±0.07	694±50.64 [c]	35.10±1.29[b]

Values are presented as mean ± SEM (n=5 animals/group)

a) Significantly different from control group (BPA with control) at ($P < 0.05$)

b) Significantly different from BPA group

c) Significantly different from control group (control with green tea groups)

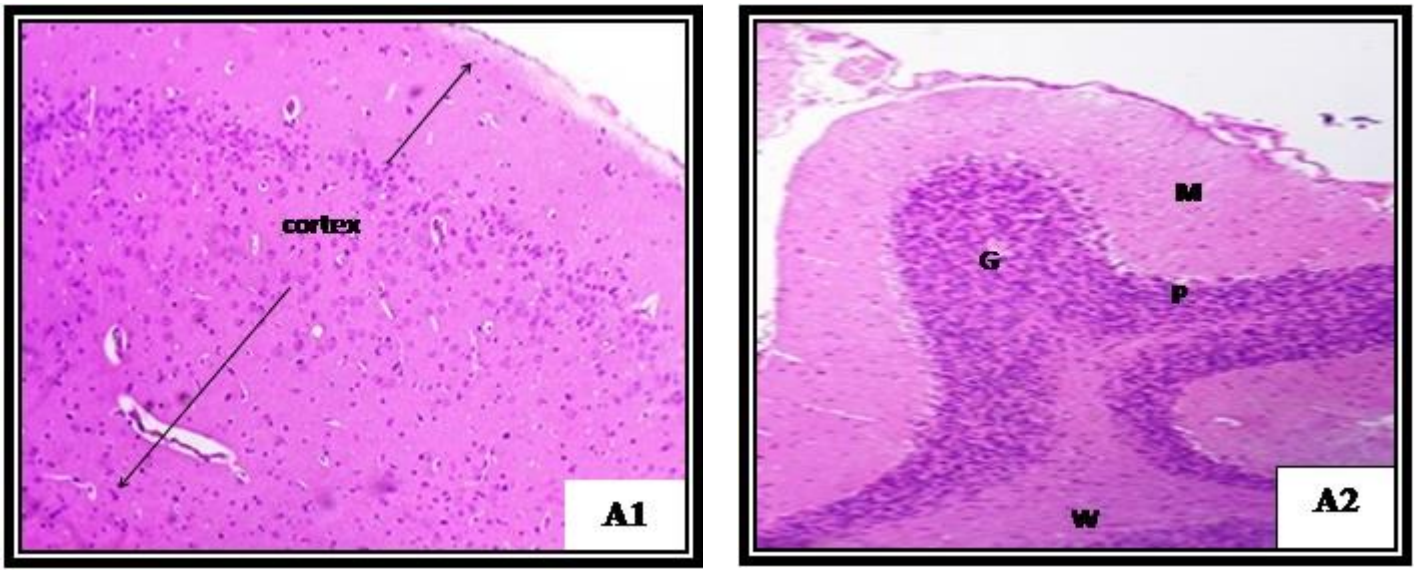
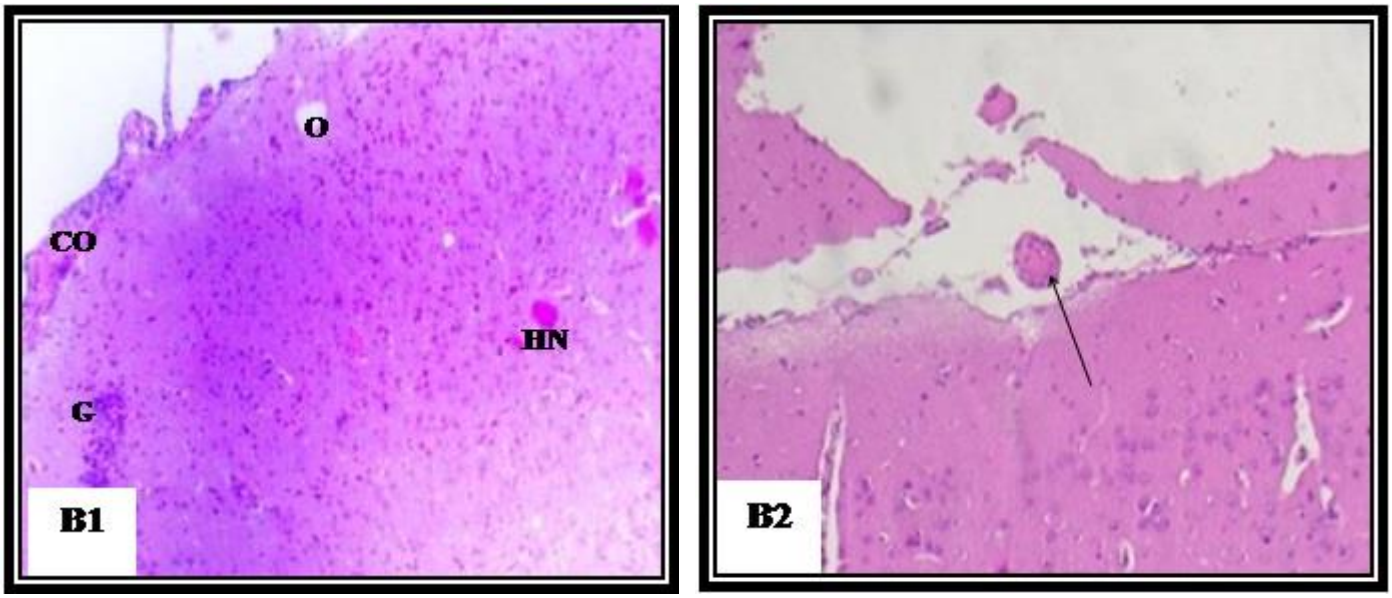


Figure 1. Cross section in the brain of control group. A1) normal cerebral cortex A2) normal cerebellum structure, granular layer (G), molecular layer (M), Purkinje cells layer (P), white matter (W).



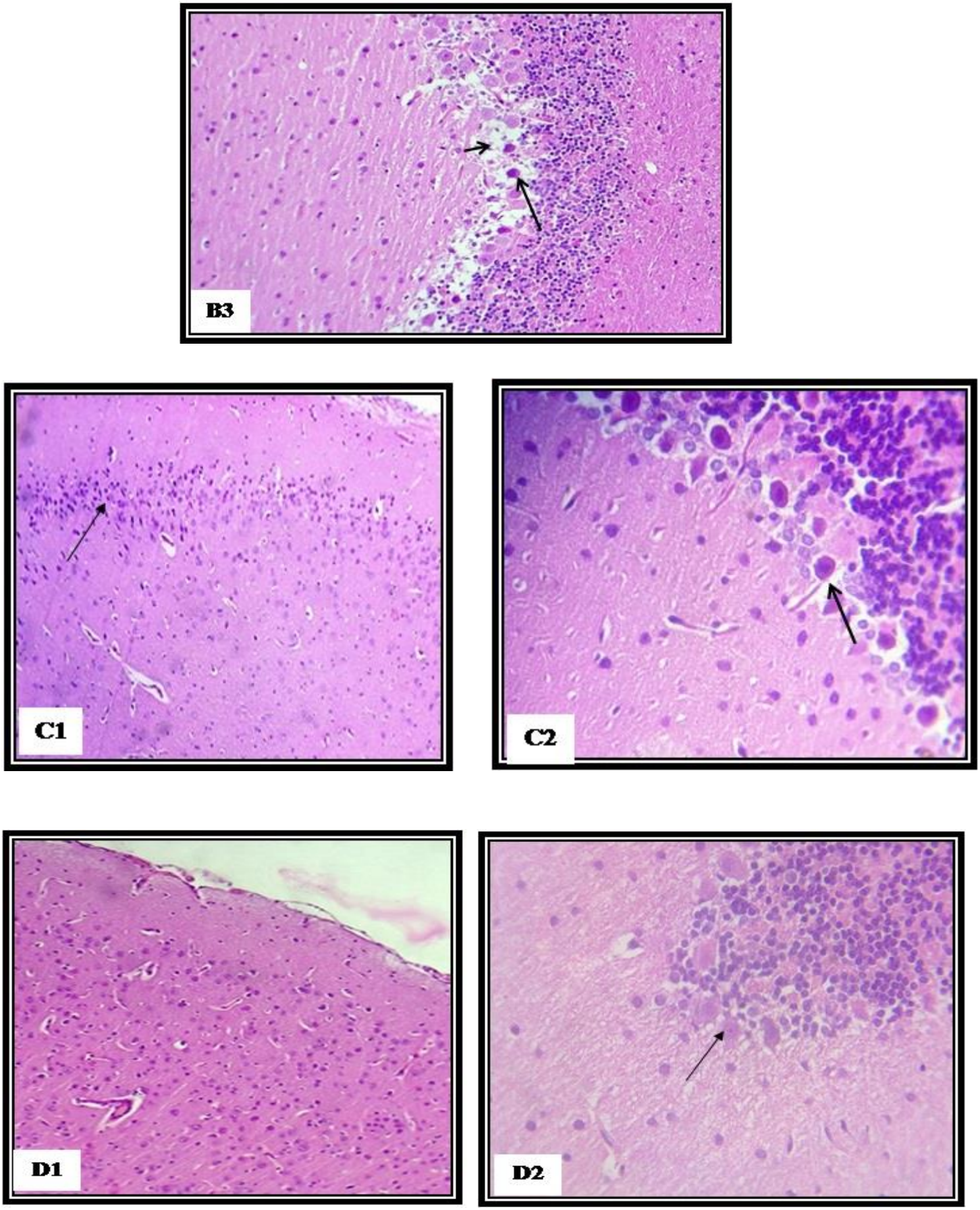
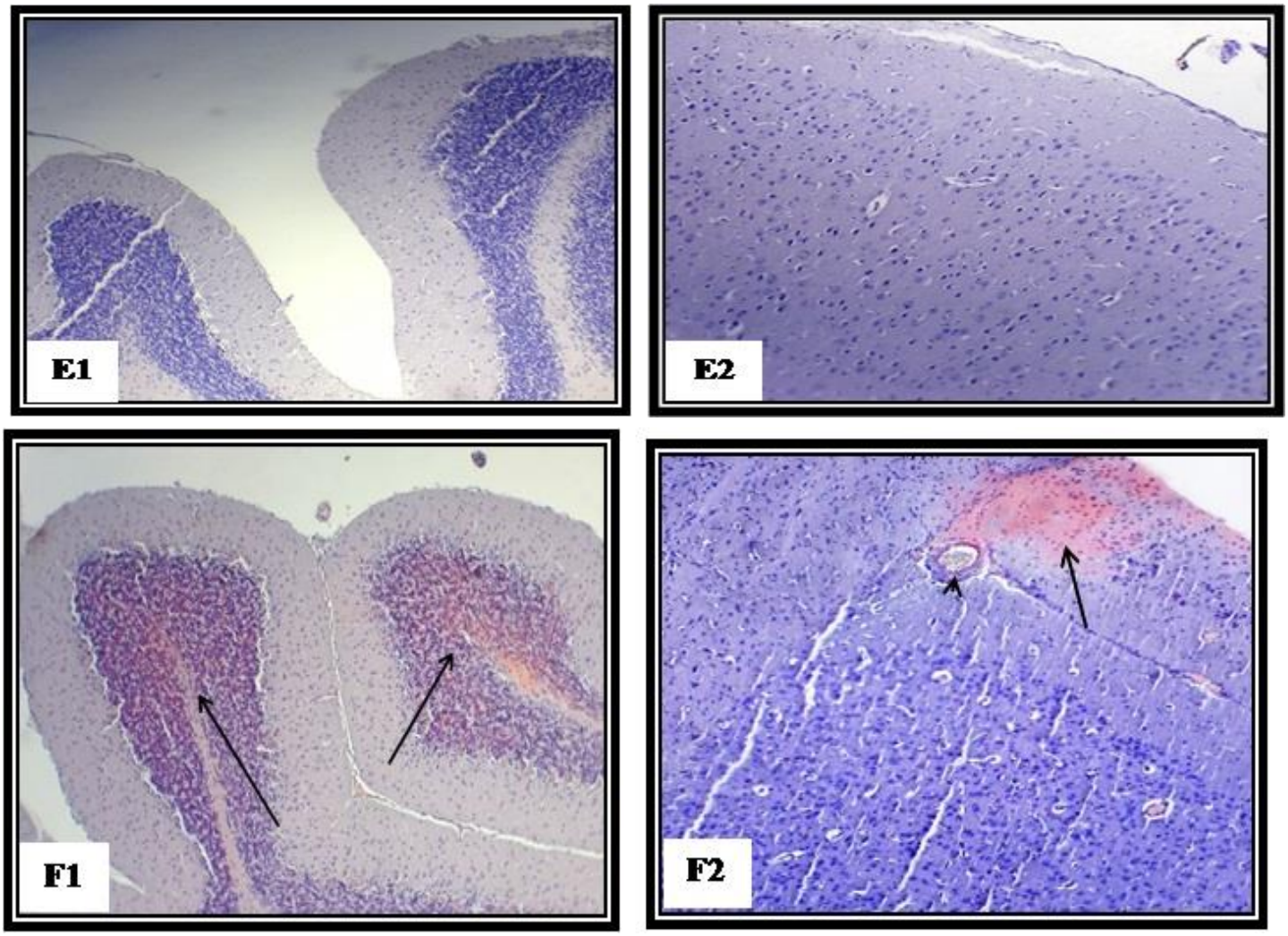


Figure 2. Cross section in the brain of the BPA treated groups B1) Cerebrum shows odema (o), Blood vessels congestion (c), Hyaline necrosis (hn), Gliosis (g) (H&E 10X). B2) Section in the cerebrum shows clot formation in the blood vessel (arrow). B3) Section in the cerebellum of BPA treated group showes

disarrangement and pyknosis of purkinje cells (H&E 10X). C1) Cerebrum of green150+BPA shows brain tissue improvement with slight gliosis and congestion in blood vessels (H&E 10X). C2) Cerebellum section shows preserved purkinje cells (H&E 10X). D1) Cerebrum section of green 300+BPA treated group shows normal cerebral cortex (H&E 10X). D2) Cerebellum of green 300+BPA treated group shows normal purkinje cells (arrow) (H&E 10X).



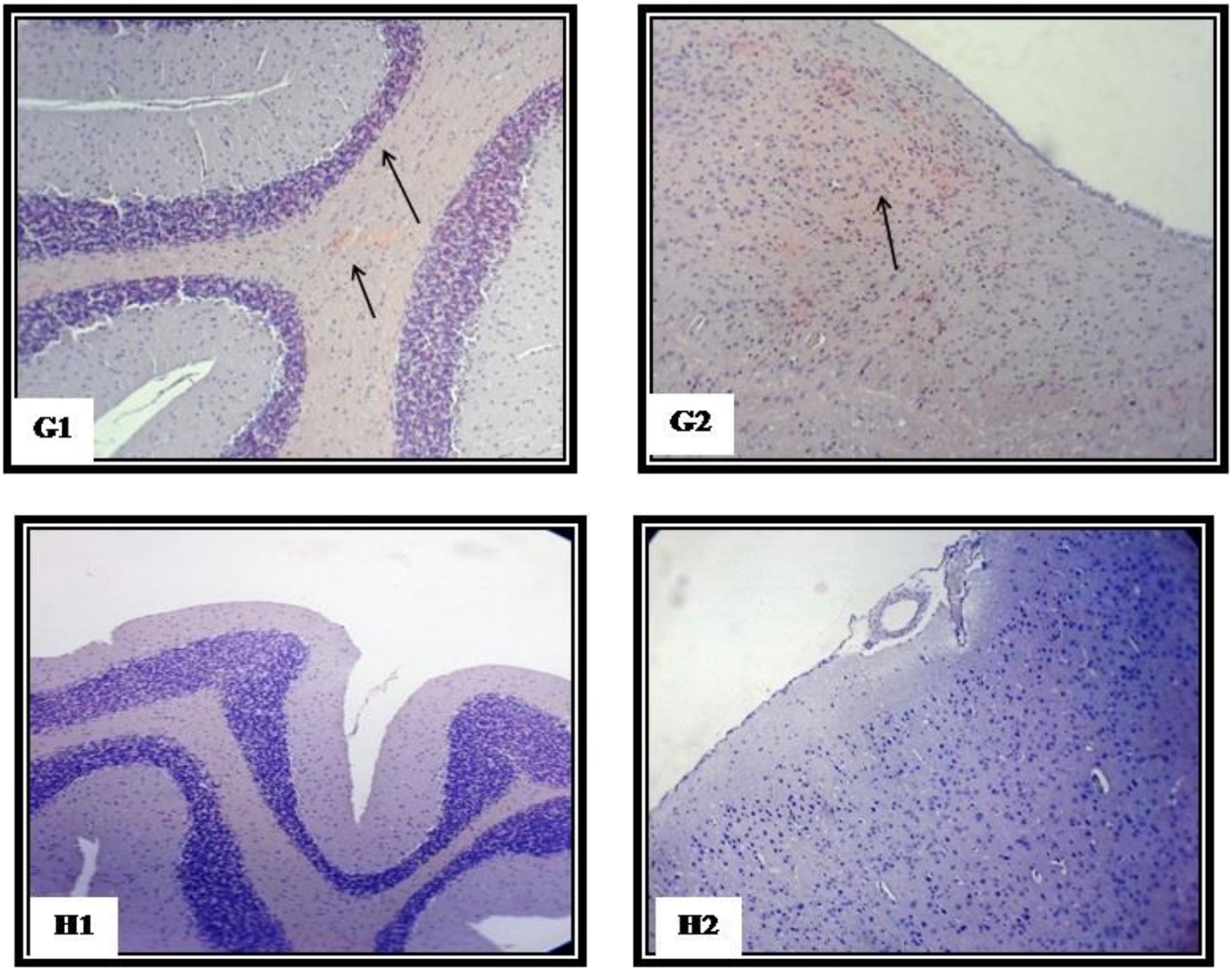


Figure3. Cross section in the brain stained with Congo red stain 10X under light microscope. E1) Normal cerebellum with no amyloid deposition in control group. E2) Normal cerebrum cortex with no amyloid deposition in control group. F1) Focal extracellular amyloid deposition (arrow) in the cerebellum taken red color under light microscope in the BPA treated group. F2) Amyloid deposition in the cerebral cortex (arrow) and wall of blood vessels (arrowhead) in BPA treated group. G1) Cerebellum of green 150+BPA treated rats shows reduced amyloid deposition. G2) Cerebrum of green 150+BPA treated rats shows reduced amyloid deposition. H1) Cerebellum of green300+BPA treated group shows no amyloid deposition. H2) Cerebrum of green300+BPA treated group shows no amyloid deposition.

4.DISCUSSION

In most cases, the etiology of BPA on the brain is completely known. Result of this study revealed a possible detrimental effect of BPA on the body and brain weight as compared to the control. The reduction in the body weight in BPA treated group may be related to the reduction in the rate of food consumption (lack of appetite); that may increase lipid mobilization and lipolysis to produce fatty acids in negative energy balance (Zechner et al., 2012). BPA has been known as xenoestrogen which works as a weak estrogen, that may alter the stimulating role of estrogen which may, in turn, increase the rate of lipolysis by suppressing food intake through a direct effect on the central nervous system (Negri-Cesi, 2015). These results concur with many previous results (2018, Perera et al., 2016). Elsewhere, some reports indicate that BPA stimulates weight gaining and cause obesity (Morgan et al., 2014).

The improvement in the final body weight and brain weight by green tea extracts may be related to the presence of antioxidant compounds catechins (GTC), which comprise four major epicatechin derivatives; namely, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG), and the extraction with ethanol ensure the highest phenolic content of the green tea extract (Setyopratomo, 2014). Other reports suggested that green tea has a lowering weight effect (Chen et al., 2011). The explanation for this disagreement with our findings, the experiment of that research was made by using healthy rats; in our study the rats were intoxicated by BPA. Related to the antioxidant properties, food intake and energy balance was improved. Body weight improvement was better seen in the higher dose of green tea with BPA. Green tea is also containing Lipids, proteins, vitamin C, and minerals (Cabrera et al., 2003).

BPA induced oxidative stress in the brain of the treated rats. Oxidative stress related to the ability of BPA to accumulate in the brain because of its lipophilic action and increase oxidative stress by increasing the activity of acetylcholinesterase in cortical and hippocampal areas of the brain (Khadrawy et al., 2016). A recent study indicated that BPA increases the gene expression of stress response genes, which increase the oxidative response and cellular damage genes (Garcia-Espineira et al., 2018). Previous studies showed that BPA has adverse effects on several organs including the developing brain, recent study indicates that BPA disrupts neural differentiation in human-derived neural progenitor cells, potentially disrupting brain development (Fujiwara et al., 2018) those changes were supposed to be mediated by stimulating oxidative damage in different organs especially for the brain (Ke et al., 2013). An earlier study showed that BPA affects CNS function in rodent and its stronger in the prenatal stage, that may be because it is easier for BPA to cross the placental barrier and blood- brain barrier (Nishikawa et al., 2010).

Our findings were shown that green tea extract was reduced oxidative stress in a dose dependent manner. Green tea extract enriched with polyphenols family (catechins) as it works as a chelating agent with high affinity for ions binding to reduce oxidative stress that mediated by low-density lipoprotein (Yoshida et al., 1999, Galleano et al., 2010, Seeram et al., 2006). The effect of green tea extracts does not appear due only for its polyphenol content, but several signaling events on cell level may be responsible for their biological actions (Mandel et al., 2004).

BPA rat exposure lead to adverse effects in the brain histology, those effects include congestion and clotting in the blood vessels of the brain, that may be related to the alteration in the concentration of some ions in the blood that contribute in blood clotting process like calcium ions, this in turn, will increase blood clotting rate (Pal et al., 2017). Besides that, BPA induced disorganization of cerebellum layers, and condensation of Purkinje cells. The changes in the brain histology that been mediated by BPA related to the oxidative stress that induced by BPA, which lead to the formation of reactive oxygen species (ROS) that may cause inflammation and cell apoptosis, finally cause neural diseases like Alzheimer disease (Mandel et al., 2004). Effect of BPA on the brain may be the same of those appeared by using pesticides (Ismail, 2017), antidepressant drugs (Rasul et al., 2016), and other plasticizers like Di-n-butylphthalate (Chawsheen and Aziz, 2013). In contrast, green tea was improved brain tissue. That related to the positive effect of flavonoids that have been attributed to the fortification of neural functioning, stimulation of neuronal recovery, and increased blood supply, and improve memory in studies that used pure flavonoids extracted from different plants (van Praag et al., 2007, Nehlig, 2013, Swinton et al., 2018).

BPA was accelerated toxic amyloid protein aggregation in the cerebrum, and cerebellum parts of the brain, obstruction in the blood vessels reduce brain blood supply, oxygen and energy and finally leading to beta- amyloid aggregation (Prasansuklab and Tencomnao, 2013). Low glucose supply to the brain induces different cellular pathways at a genetic level, resulting in overproduction of amyloid beta- protein (Bell et al., 2009). Increasing oxidative stress in the brain may be as a result of amyloid aggregation and cell damage (Behl et al., 1994). Our results come in agreement with previous studies on different organs. Gong et al. (2013) found that BPA increases the incidence of Diabetes type 2 by

increasing the accumulation of amyloid in pancreatic beta cells. A study conducted on the brain of offspring males BPA was found to increase amyloid precursor protein (Fang et al., 2016b). That may be explained by the inhibition of membrane integral protease enzyme, that is responsible for the degradation of amyloid precursor protein (Baba et al., 2009).

Our data showed that green tea extracts reduced amyloid aggregation in the brain of the treated rats. That may be related to the inhibitory effect of green tea catechins for amyloid fibril formation (aus dem Siepen et al., 2015) (Rezai-Zadeh K et al., 2005). These results came in agreement with previous studies (Mereles et al., 2008). The possible explanation that green tea catechins were worked in contrast with BPA by stimulating the alpha-secretase enzymes that are responsible for the transforming of amyloid precursor protein into non-amyloid protein rather than toxic beta - amyloid (Obregon et al., 2006).

CONCLUSION

BPA induced oxidative stress may be mediated by amyloid beta -protein accumulation. Green tea extracts ameliorate BPA neural changes by reducing oxidative stress and have a neuro-protective effect against brain amyloid toxicity.

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Conflict of interest: there is no conflict of interest.

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