



Effects of Hydro-ethanolic Extract of *Tanacetum parthenium* and its N-Butanol and Aqueous Fractions on Brain Oxidative Damage in Pentylenetetrazole-Induced Seizures in Mice

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Abstract

Background: Epileptic seizures affect the life of noticeable number of people in all over the world. *Tanacetum parthenium* (TP) is used in traditional medicine. We studied the effects of hydro-ethanolic extract of TP and its n-butanol and aqueous fractions on brain oxidative damage in pentylenetetrazole (PTZ)-induced seizures in mice.

Methods: Male mice were divided into: (1) Control; (2) PTZ (100 mg/kg, i.p.); (3-5) hydro-ethanolic extract of TP (50, 100 and 200 mg/kg); (6) n-butanol (NBut) (100 mg/kg) and (7) aqueous (Aq) (100 mg/kg) fractions. Extracts were injected (i.p.) for 3 days and 30 min before PTZ. Latencies in onset of Minimal Clonic Seizures (MCS) and Generalized Tonic-Clonic Seizures (GTCS) as well as biochemical indicators were evaluated.

Results: Medium dose of TP extract and NBut fraction prolonged the MSC and GTCS latencies. Biochemical data confirmed that administration of hydro-ethanolic extract of TP significantly reduced MDA and enhanced total thiol content and the activity of SOD and CAT in brain tissues. Comparison the effect of NBut and Aq fractions with medium dose indicated a higher level of MDA and lower amount of total thiol content and the activity of SOD and CAT in brain tissues of PTZ-Aq100 and PTZ-NBut100 groups than PTZ-TP100 group.

Conclusion: Results demonstrated that the medium dose of TP extract had the most protective effect against brain oxidative damage in PTZ-induced seizure model. N-butanol and aqueous fractions of TP could not exert stronger effect than medium dose on reduction PTZ-induced brain oxidative stress.

Introduction

Epileptic seizures affect the life of noticeable number of people in all over the world.¹ In epileptic seizures the sudden discharge of neurons are associated with muscle contraction² and cognitive behaviors dysfunction.³ Oxidative stress,⁴ inflammation,⁵ programmed cell death⁶ and heat shock proteins⁷ have been suggested as important causes in epileptic seizures pathogenesis. Dysfunction of membrane proteins including neurotransmitter receptors and ion channels followed by brain oxidative and nitrosative stress has been also demonstrated to involve in neuronal hyper-excitability.^{8,9} In addition, the elevated level of inflammatory cytokines such as interleukin (IL) 1 β in

the brain has been reported to promote seizure activities.¹⁰ Electrical¹¹ and chemical¹² kindling are employed for induction of epileptic seizures in experimental models. Kainic acid¹³ and pentylenetetrazole (PTZ)¹⁴ are two well-known chemical substances which are applied for seizure outspread in laboratory animals. PTZ as a GABA_A receptors blocker has been shown to decrease the level of antioxidant indexes such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) in cells of CA1 and CA3 areas of hippocampus in rats.¹⁵ Researchers also reported that the activation of glucagon like peptide 1 receptor (GLP1R) associated with inhibition of oxidative

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stress and up-regulation of heat shock protein70 (Hsp70) in hippocampus of rats could exert the protective effects against PTZ-stimulated seizures.¹⁶ In addition, increased level of pro-inflammatory mediators such as IL-1 β , IL-6, IL-18 and tumor necrosis factor- α (TNF- α) was reported in rats kindled by PTZ.¹⁷

Tanacetum parthenium (TP), named feverfew, is a member from Asteraceae family which grows in different areas of the world such as Iran.¹⁸ Therapeutic effects including anti-inflammatory,¹⁹ anti-asthma,¹⁸ anti-fever,²⁰ anti-migraine and anti-arthritis²¹ are attributed to this plant. Antioxidant ingredients such as sesquiterpene lactones, parthenolide, flavonoids and luteolin have been also identified in extract of TP.^{22,23} It has been documented that pre and post treatment with TP extract ameliorated hepatotoxicity followed by CCl₄ injection via reduction of free radicals, modulation of antioxidant enzymes activity such as superoxide dismutase (SOD) and catalase (CAT) and suppression of lipid peroxidation in rats.²⁴ Researchers showed that the quercetin present in TP extract has considerable protective effect on central nervous system.²⁵ This substance derived from TP extract also could enhance slow waves sleep in rats.²⁶ TP extract and its constituents have been also suggested to alleviate the headaches resulted from migraine through inhibition of nitric oxide production, suppression of serotonin release and reduction of Calcitonin Gene-Related Peptide (CGRP) secretion.²⁷ Despite these lucrative properties, the anticonvulsant effect of TP has been not understood. Therefore, we designed this study to assess the effect of TP extract and its N- butanol and aqueous fractions on brain oxidative damage in PTZ-induced seizures in mice.

Materials and Methods

Drugs and chemicals

PTZ and biochemical reagents were used. PTZ was prepared from Sigma Aldrich Company [St. Louis, USA]. Reagents were bought from Merck Company.

Preparation of the plant extract and the fractions

Using a blender, the plant material was grounded to fine powder. To prepare the hydro-ethanolic extract of TP, 50 g of the provided powder was extracted during 48 h with 300 ml ethanol-water (70/30 v/v) using a Soxhlet apparatus. The solvent was removed using vacuum distillation method. The yielded of this extract was 10% (w/w). To prepare the fractions, each 10 g of dried hydro-ethanolic extract was suspended in 300 ml distilled water, transferred to a separator funnel, and partitioned with n-butanol (300 mL \times 6). The N-butanol fraction was separated and the lower water-soluble layer was considered as water fraction.^{14,28} The resulting fractions were dried on a water bath. Water fraction dissolves polar compounds and water-soluble plant constituents. N-butanol fraction has non-polar agents.²⁹

Animals and groups

Male mice were randomly distributed into 7 groups (n=10 mice/group): (1) Control group, received saline instead of PTZ and extract, (2) PTZ group, received 100 mg/kg of PTZ, (3-5) groups, given 3 doses (50, 100 and 200 mg/kg)²⁹ of hydro-ethanolic extract of TP (6) n-butanol (NBut) group, treated with 100 mg/kg of n-butanol fraction of TP and (7) Aqueous (Aq) group, injected with 100 mg/kg of aqueous fraction of TP. In 3-7 groups, the extract and the fractions were intraperitoneally administered for 3 days and 30 minutes before PTZ injection.

PTZ-induced seizures

Induction of seizure in mice was carried out by PTZ. For this purpose, after administration of PTZ (100 mg/kg) the animals were located in a plexiglass chamber (30 \times 30 \times 30 cm) and their behavior was monitored for 60 min. The latency time in onset of Minimal Clonic seizure (MCS) and Generalized Tonic-Clonic seizure (GTCS) were considered as indexes of seizures intensity.

Biochemical measurement

After behavioral tests, the animals were euthanized and their brains were collected for biochemical measurements.

Assessment of malondialdehyde concentration

The assessment of malondialdehyde (MDA) concentration was done using relevant reagents as it has been previously declared.³⁰

Determination of total thiol content

The total thiol content (SH) was evaluated using appropriate reagents as we have already described.³⁰

Estimation of SOD activity

The activity of SOD was estimated based on the method of Madesh and Balasubramanian as we have depicted it in our previous works.³¹

Measurement of CAT activity

The activity of CAT was measured using the method of Aebi as it has been previously explained.³²

Statistical analysis

The findings were analyzed using the SPSS 20 software. The groups were compared with each other by one way ANOVA test followed by LSD as the post hoc test. P <0.05 was considered to be statistically significant.

Results

Effect of hydro-ethanolic extract of TP on PTZ-induced seizures

Administration of PTZ triggered the MCS and GTCS in all groups. The medium dose (100 mg/kg) of hydro-ethanolic extract of TP lengthened the onset of seizure attacks (MCS and GTCS) in PTZ-TP100 group than PTZ group (P<0.001). Based on results, the latency time in onset of MCS

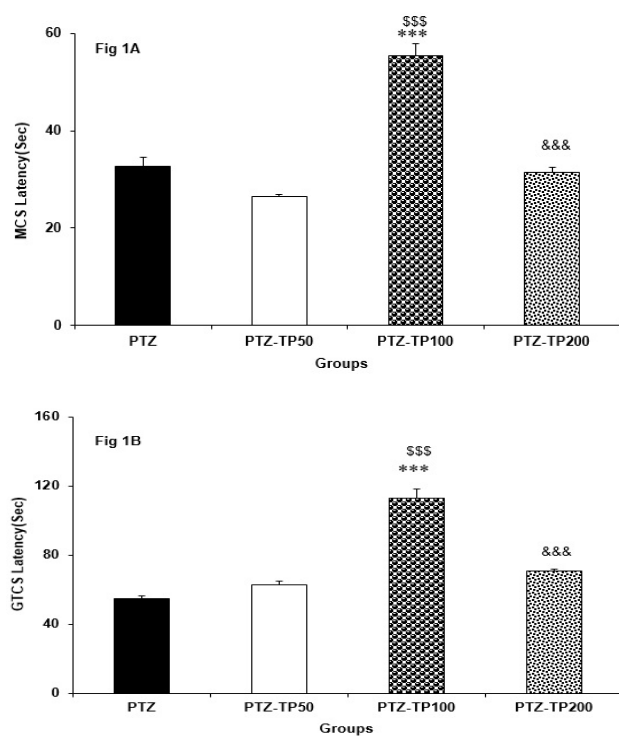


Figure 1. Comparison the effect of TP extract on latency time in beginning of minimal clonic seizures (MCS) (A) and generalized tonic-clonic seizures (GTCS) (B) in mice. Data were exhibited as the mean \pm SEM (n= 10). ***P<0.001 versus the PTZ group, ^{SSS}P<0.001 versus PTZ-TP50 and ^{&&&}P<0.001 versus PTZ-TP100.

and GTCS in PTZ-TP100 group was more than PTZ-TP50 group (P<0.001). The delay in onset of MCS and GTCS in PTZ-TP200 group was also lesser than PTZ-TP100 group (P<0.001). Data did not exhibit any significant difference in onset of MCS and GTCS between PTZ-TP50 and PTZ-TP200 groups with PTZ group (Figures 1A and 1B).

Comparison the effect of the most effective dose (100 mg/kg) of TP extract with its aqueous and N- butanol fractions on PTZ-induced seizures

Pretreatment with n-butanol fraction of TP postponed the onset of MCS and GTCS in PTZ-NBut100 group compared with PTZ group (P<0.001). We did not perceive any significant difference in onset of MCS and GTCS in PTZ-NBut100 group with respect to PTZ-TP100 group. According to the results of current study, the effect of aqueous fraction of TP on latency time of seizure parameters was lesser than that of PTZ-TP100 group (P<0.01). Data also denoted that the beginning of MCS and GTCS in PTZ-NBut100 group was longer than PTZ-Aq100 group (Figures 2A and 2B).

Effect hydro-ethanolic extract of TP on oxidative stress indicators in cortical and hippocampal tissues

We observed an increased accumulation of MDA in cortical and hippocampal tissues of PTZ group than control group (P<0.001). All three doses of TP extract remarkably lowered the concentration of MDA in brain tissues compared with PTZ group (P<0.001). MDA level of injected mice with

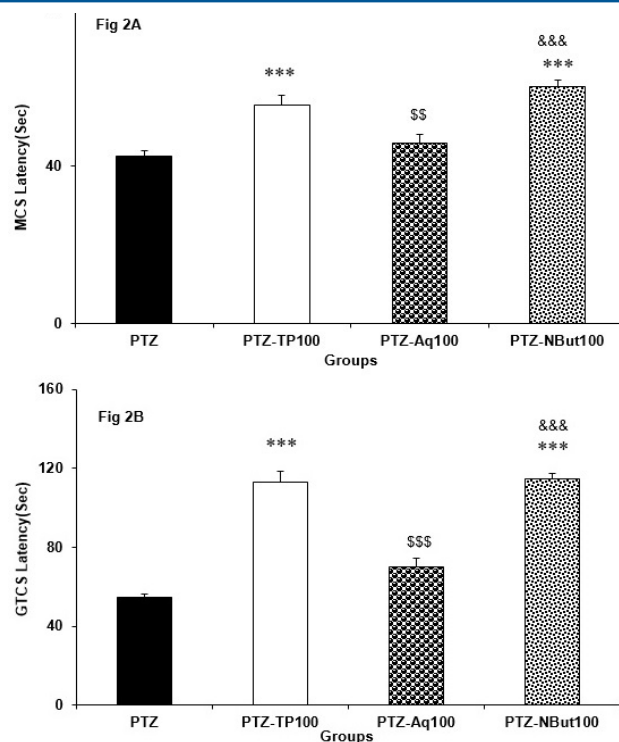


Figure 2. Comparison the effect of medium dose of TP extract with its aqueous and N-butanol fractions on latency time in beginning of minimal clonic seizures (MCS) (A) and generalized tonic-clonic seizures (GTCS) (B) in mice. Data were presented as the mean \pm SEM (n= 10). ***P<0.001 versus the PTZ group, ^{SS}P<0.01 and ^{SSS}P<0.001 versus PTZ-TP100 and ^{&&&}P<0.001 versus PTZ-Aq100.

100 mg/kg of TP extract was noticeably lower than those treated with 50 mg/kg of TP extract (P<0.01). Data did not indicate any significant difference between PTZ-TP100 and PTZ-TP200 groups in this parameter (Figures 3A and 4A). The total thiol content was also assessed in all experimental groups. We figured out a significant reduction in concentration of this antioxidant indicator in brain tissues of treated mice with PTZ in comparison with control group (P<0.001). The hippocampal tissue results demonstrated that all three doses of TP extract raised the total thiol level of PTZ-TP groups than PTZ group (P<0.01 and P<0.001). According to the cortical tissue data, only the dose of 100 mg/kg of TP extract could significantly enhance the total thiol content in comparison with PTZ group (P<0.001) (Figures 3B and 4B). As clarified in Figures 3B and 4B, the medium dose of TP extract (100 mg/kg) significantly increased the total thiol level of mice brain tissues than dose 50 mg/kg (P<0.01 and P<0.001). Findings also showed that the total thiol content in brain tissues in PTZ-TP200 group was lower than PTZ-TP100 group (P<0.05 and P<0.001) (Figures 3B and 4B).

We also checked the activity of SOD and CAT in brain tissues of mice. The results demonstrated that the activity of these antioxidant enzymes in cortical and hippocampal tissues of mice was lower in PTZ group than control group (P<0.001). All three doses of TP extract elevated the SOD activity in hippocampal and cortical tissues of PTZ-TP groups than PTZ group. The activity of CAT in

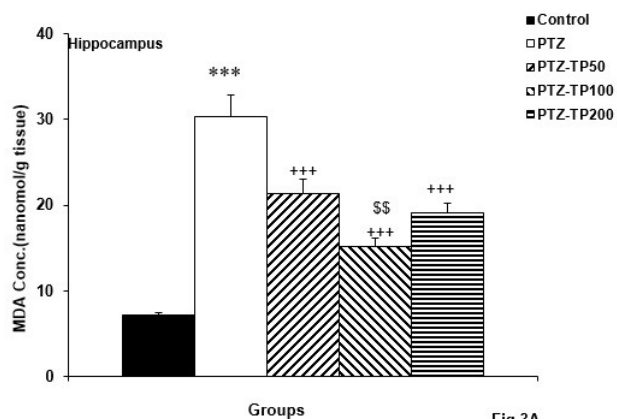


Fig 3A

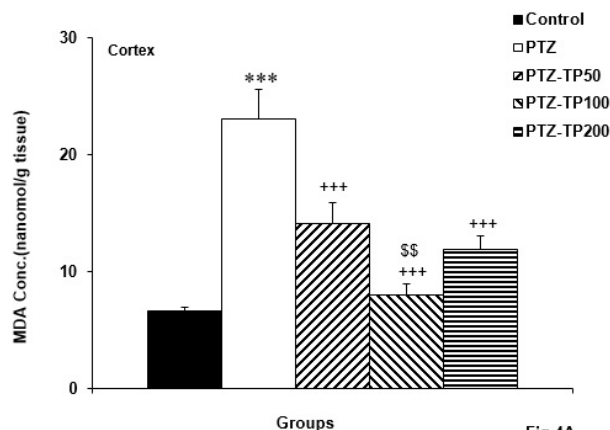


Fig 4A

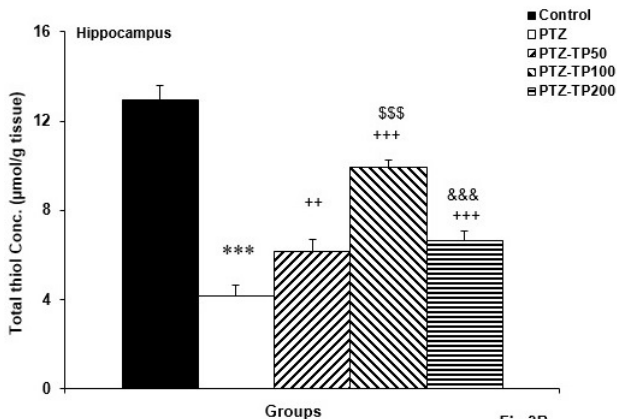


Fig 3B

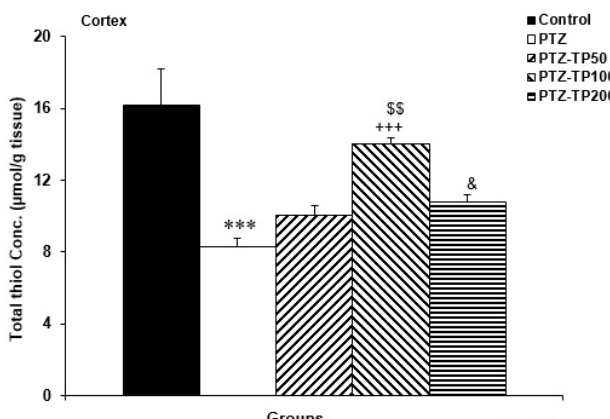


Fig 4B

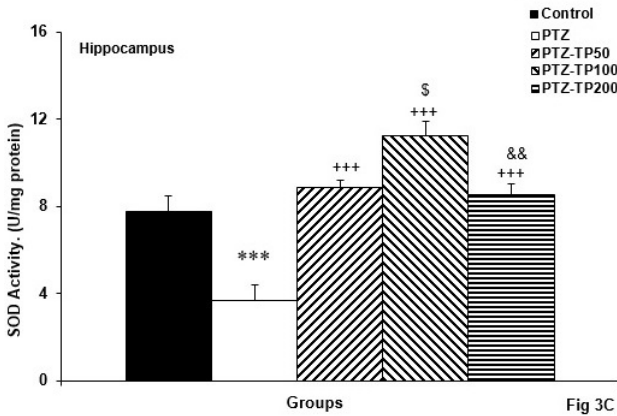


Fig 3C

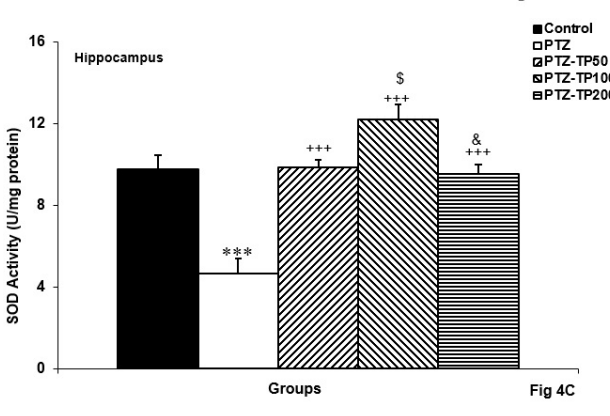


Fig 4C

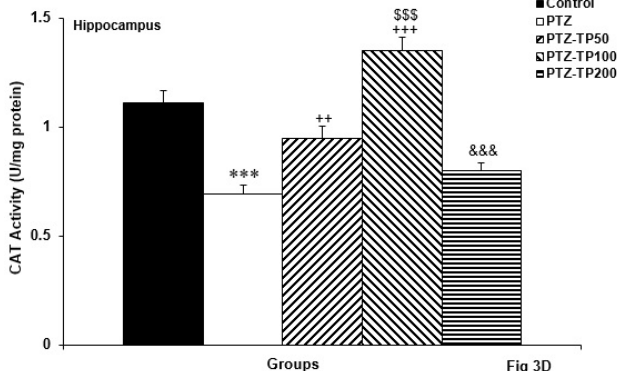


Fig 3D

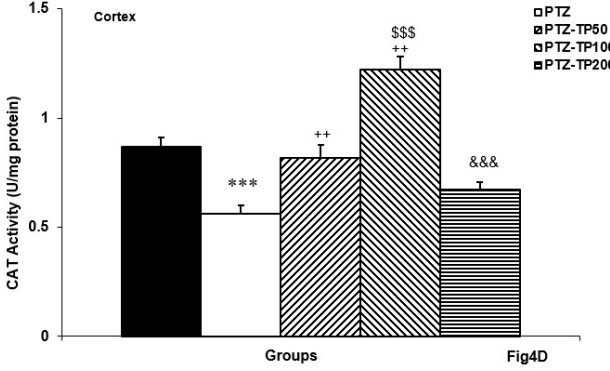


Fig 4D

Figure 3. Comparison of MDA concentration (A), total thiol level (B) and activity of SOD (C) and CAT (D) in hippocampus tissue in treated groups. Data were declared as the mean \pm SEM (n= 10). ***P<0.001 versus control group, **P<0.01 and ***P<0.001 versus PTZ group, ^sP<0.05, ^{ss}P<0.01 and ^{sss}P<0.001 versus PTZ-TP50 group and ^{&&}P<0.01 and ^{&&&}P<0.001 versus PTZ-TP100.

Figure 4. Comparison of MDA concentration (A), total thiol level (B) and activity of SOD (C) and CAT (D) in cortical tissue in treated groups. Data were declared as the mean \pm SEM (n= 10). ***P<0.001 versus control group, **P<0.01 and ***P<0.001 versus PTZ group, ^sP<0.05, ^{ss}P<0.01 and ^{sss}P<0.001 versus PTZ-TP50 group and [&]P<0.05 and ^{&&}P<0.001 versus PTZ-TP100.

PTZ-TP50 and PTZ-TP100 groups also was higher than PTZ group ($P < 0.01$ and $P < 0.001$). The results confirmed that the activity of SOD and CAT in hippocampal and cortical tissues of PTZ-TP100 group was more than PTZ-TP50 ($P < 0.05$ and $P < 0.001$). The activity of SOD and CAT in mice brain tissues in PTZ-TP200 group was lesser than PTZ-TP100 group ($P < 0.05$, $P < 0.01$ and $P < 0.001$) (Figures 3C, 3D, 4C and 4D).

Comparison the effect of the most effective dose (100 mg/kg) of TP extracts with its aqueous and N- butanol fractions on oxidative stress indexes

Biochemical results displayed that the MDA level of hippocampal and cortical tissues of PTZ-Aq100 and PTZ-NBut100 groups was higher than PTZ-TP100 group ($P < 0.001$) (Figures 5A and 6A). The total thiol level of brain tissues of PTZ-Aq100 group was lesser than PTZ-TP100 group ($P < 0.01$). The content of total thiol of hippocampal tissue of PTZ-NBut100 group was more than PTZ-Aq100 group ($P < 0.01$). There was not any significant difference in total thiol level of brain tissues of PTZ-NBut100 group with PTZ-TP100 group (Figures 5B and 6B). Data indicated that the activity of SOD and CAT in hippocampal and cortical tissues of PTZ-Aq100 and PTZ-NBut100 groups was lower than PTZ-TP100 group ($P < 0.01$ and $P < 0.001$) (Figures 5C, 5D, 6C and 6D).

Discussion

Neuronal hyper-excitability resulted from repeated seizures can terminate to neuro-degeneration and brain oxidative damage.³³ PTZ is a GABA_A receptors inhibitor which can irritate frequent neuronal discharges resulting in convulsion and neuronal death.^{34,35} In present work, intraperitoneal injection of PTZ led to severe neuronal discharges associated with fast MCS and GTCS in mice. Oxidative stress,⁴ inflammatory responses¹⁰ and apoptosis⁶ have been proposed to have an important contribution in brain seizures pathogenesis. Down-regulation of antioxidant agents such as total SOD, α - tocopherol and glutathione disulfide (GSSG) in cerebral cortex of PTZ-kindled animals has been also documented.^{33,36} Electron paramagnetic resonance (EPR) imaging from brain of mice kindled by PTZ indicated a strong imbalance in oxidative state in susceptible brain areas to oxidative damage including cortex and hippocampus.³⁷ Moreover, uncontrolled generation of free radicals such as nitric oxide (NO) and mitochondrial damage in epileptic brain has been revealed.³⁸ In agreement with these reports, we observed an imbalance in oxidative state of cortex and hippocampus tissues of PTZ-kindled mice. Higher level of MDA, lower content of thotal thiol groups and lesser activity of antioxidant enzymes, SOD and CAT, in cortex and hippocampus tissues of PTZ-injected mice than those

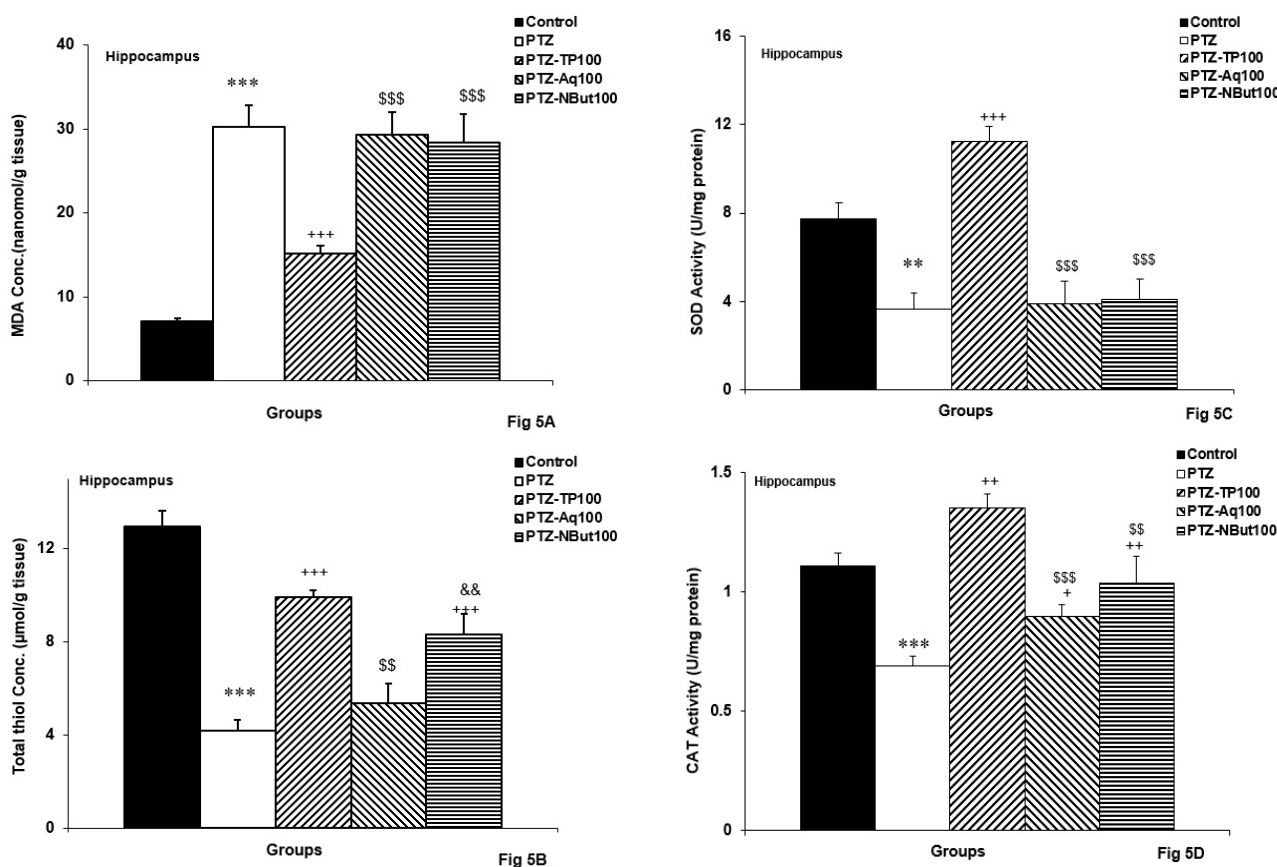


Figure 5. Comparison the effect of aqueous and N-butanol fractions of TP with medium dose on MDA concentration (A), total thiol level (B) and activity of SOD (C) and CAT (D) in hippocampus tissue in treated groups. Data were showed as the mean \pm SEM (n= 10). *** $P < 0.001$ versus control group, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ versus PTZ group, $^{ss}P < 0.01$ and $^{sss}P < 0.001$ versus PTZ-TP100 group and $^{&P} < 0.01$ versus PTZ-Aq100.

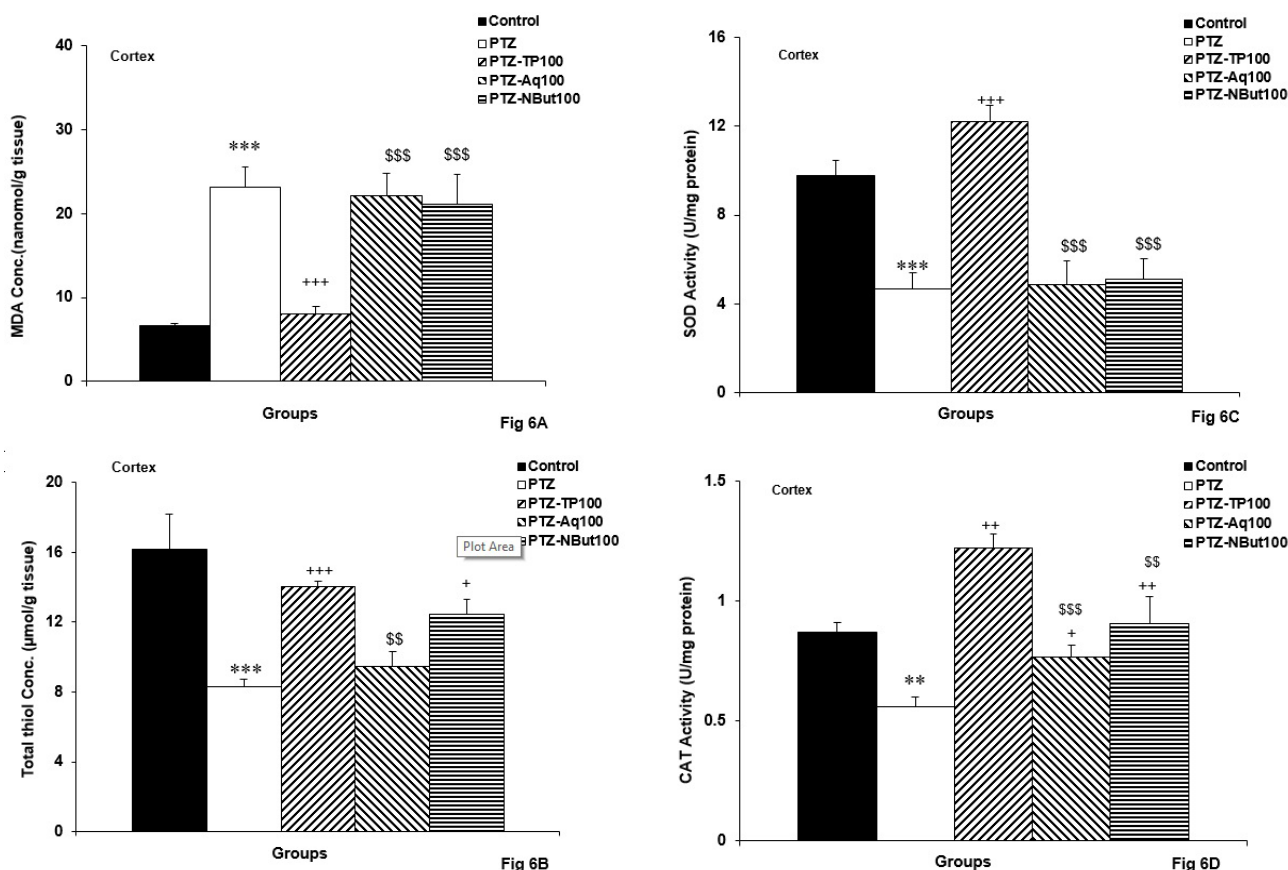


Figure 6. Comparison the effect of aqueous and N-butanol fractions of TP with medium dose on MDA concentration (A), total thiol level (B) and activity of SOD (C) and CAT (D) in cortical tissue in treated groups. Data were showed as the mean \pm SEM (n= 10). **P<0.01 and ***P<0.001 versus control group, **P<0.01 and ***P<0.001 versus PTZ group, \$\$P<0.01 and \$\$\$P<0.001 versus PTZ-TP100 group.

of control group support this scientific finding. TP is a medicinal herb which has an abroad spectrum of therapeutic properties including anti-inflammatory,¹⁹ anti-asthma,¹⁸ anti-pyrogenic,²⁰ anti-migraine,³⁹ anti-arthritis,⁴⁰ anti-parasitic²⁰ and anti-diabetic.⁴¹ In this study, to determine the most effective dose we examined the effect of three doses of hydro-ethanolic extract of this plant on PTZ-promoted seizures in mice. Retardation in inception of MCS and GTCS is considered as an index to evaluate the violence of seizures in experimental models.²⁹ According to behavioral results; the moderate dose of TP extract (100 mg/kg) acted as the best dose and prolonged the beginning of MCS and GTCS in animals of PTZ-TP100 group compared with PTZ group. Our results indicated that the lowest and highest doses of extract did not affect the delay in onset of seizures caused by PTZ injection. Therefore, it is deduced that the effect of TP extract on PTZ-induced seizures was applied in dose-independent manner in rats. In traditional medicine, there are a large massive of evidence that emphasize that the plant extracts and their ingredients can improve brain oxidative stress.^{42,43} Parthenolide,²² luteolin and flavonoids²³ found in TP extract has been introduced to be as antioxidant compounds. Mitigation of free radicals accumulation and augmentation of SOD and CAT by TP extract in CCl₄-caused hepatotoxicity in rats has been illustrated.²⁴ In another study, researchers

suggested that methanolic extract of TP improved renal dysfunction induced by CCl₄ in rats. This ameliorative effect was attributed to decline in lipid peroxidation and enhancement of SOD and GPx activity in renal tissues of rats.⁴⁴ In current study, the moderate dose of TP normalized oxidative stress state in cortical and hippocampal tissues of mice. Decrement in accumulation of MDA, increment in total thiol level and amplification in SOD and CAT activity were perceived in cortical and hippocampal tissues of PTZ-TP100 group than the animals of PTZ group. Thus it seems that a part of anticonvulsant effects of TP that perceived in current investigation was carried out via modulation of oxidative damage resulted from PTZ administration. Furthermore, parthenolide present in TP extract has been suggested to attenuate inducible nitric oxide synthase (iNOS) expression, to reduce the activity of transcriptional causes involved in inflammatory pathways such as nuclear factor- κ B and to inhibit I κ B kinase complex β as one of the principal player in inflammation caused by cytokines.²⁷ Based on these findings, modulation of inflammatory responses by anti-inflammatory causes of TP extract can be considered as another possible mechanism for anticonvulsant effects of this herb. However; this needs to more detailed investigation to elucidate. Recently, hypnotic potencies of hydro-alcoholic extract and ethyl acetate fraction of TP have been confirmed in rats.

Scientific studies also showed that ethyl acetate fraction which possesses ingredients with partial polarity such as flavonoids not only shortened latency of sleep but also lengthened the time of sleep in rats.⁴⁵ Hydro-ethanolic fraction of TP has been also reported to have antiviral effects. This antimicrobial effect is due to parthenolide and chlorogenic present in this fraction.⁴⁶ In current study we also compared the effect of n-butanol and aqueous fractions of TP with its moderate dose of TP extract on seizure parameters and biochemical dials. Based on behavioral results, the effect of n-butanol fraction in putting off the latency time of MCS and GTCS irritated by PTZ was similar to that of moderate dose. As shown in Figures 2A and 2B the administration of aqueous fraction of TP did not affect the latency time of MCS and GTCS in PTZ-Aq100 group compared with PTZ group. Biochemical findings also demonstrated that neither n-butanol fraction nor aqueous fraction could exert a better effect on brain oxidative damage followed by PTZ infusion than moderate dose of TP. Therefore, it was deduced that effective ingredients of TP extract are likely not purified by n-butanol and aqueous solvents. We also compared the effect of n-butanol fraction of TP with its aqueous fraction on PTZ-induced seizures. Findings showed that n-butanol fraction of TP with respect to aqueous fraction considerably could attenuate the severity of seizures. Considering the extraction of polar ingredients by water fraction and dissociation of non-polar agents by n-butanol fraction,²⁹ it is possible that non-polar compounds contribute in reduction of seizures in our study. Unlike the behavioral results we did not find any significant difference in concentration of biochemical indexes in brain tissues of mice between these two fractions. Therefore, it was concluded that likely other mechanisms are responsible in reduction seizures by TP fractions. But it needs to be examined more precisely in future.

Conclusion

In final, the results showed that the moderate dose of hydro-ethanolic extract of TP acted as the most effective dose and diminished the severity of seizures triggered by PTZ in mice. These effects can be attributed to probable antioxidant properties this extract. In addition, it was detected that n-butanol and aqueous fractions of TP could not restore brain oxidative stress caused by PTZ when they compared with moderate dose.

Ethical Issues

All processes of this project were confirmed by the research ethics committee of Mashhad University of Medical Sciences with IR.MUMS.fm.REC.1397.134 ethic code.

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Conflict of Interests

The authors claim that there is no conflict of interest.

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