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THE EVALUATION OF HISTOLOGICAL CHANGES AND IMUNOHISTOCHEMICAL EXPRESSION OF AMYLOID PRECURSOR PROTEIN IN CEREBRAL AND CEREBELLAR CORTICES IN NEWBORN MICE AFTER PRENATAL EXPOSURE TO TRAMADOL

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ABSTRACT

Tramadol is a centrally acting analgesic that used to reliefmodereate to severe pain, its use in pregnancy is generally avoided, still little information available on its effect on the central nervous system development if taken during pregnancy. Amyloid precursor protein(APP) is expressed in many tissues including neurons, it play a major role in neuronal survival, synapse formation, and neuronal growth. This study implicated to demonstrate the changes in APP expression in cerebral and cerebellar cortices upon perinatal exposure to different doses of tramadol, with its effect on their normal histological features. A sample of 60 pregnant mice were divided into four groups (15 in each group), control group that received D.W daily intra peritoneally, and test groupssubdivided into (A,B,C) those received 40,60, and 80mg/kg of tramadoldaily intra peritoneally from the start of pregnancy. Cerebral and cerebellar cortices of one day newborn mice were prepared for paraffin sections for H & E, and anti- APP ab15272 imunohistochemical stain that assessed by Aperio scope image analysis software V9. Significant changes in histological features were seen include cerebral and cerebellaratrophy, vascular changes (dilatation and rupture of vessels, cortex hemorrhagic areas), apoptotic changes, changes in cellular layers of pyramidal cells in cerebral cortex and loss of purkinji cells layer in cerebellar cortex, also changes in choroid plexus vascular pattern of the 4th ventricle. APP protein expression showed significant changes among groups (assessed by ANOVA) and between groups (assessed by un-paired t-test) compared to control, except

between group A versus control in cerebral cortex, and between control and group C of test groups, group B of test groups versus group C, and group B and C of test groups in cerebellar cortexat $p \leq 0.05$. These findings indicate the harmful effect of perinatal tramadol on the cerebral and cerebellar development specifically in higher doses, and this is associated with increase in APP expression which is proportional to the dose of treatment in a way to protect neuronal tissue and to trigger apoptosis.

KEYWORDS: Cerebral Cortex, APP, Apoptosis.

INTRODUCTION

Tramadol is a centrally acting weak opioid analgesic drug that is used in the management ofmoderate to severe pain.[1,2] Experimental data suggest that tramadol exerts its analgesic effect through the activation of the central inhibitory monoaminergic pathway.[3] Unlike typical opioids, it has low potential for dependency, tolerance and drug abuse.[4, 5] However, physical dependence and abuse of tramadol have also been reported.[6,7] Tramadol use in pregnancy is generally avoided as it may cause some reversible withdrawal effects in the newborn. A small prospective study reported an increased risk of miscarriages, with no major malformations reported in the newborn.[8]

APP is a type of trans-membrane protein with a large N-terminalextracellular domain, and a short cytoplasmic domain. APP undergoes multiple proteolytic steps, includecleavage by either α- or β-secretase releases the large APP ectodomain, sAPPα and sAPPβ respectively, or called (luminal domain); leaving the membrane anchored C-terminal fragments (CTFs).[9] Itsexpressed in many tissues including the brain and spinal cord (central nervous system)by the APP gene and concentrated in thesynapses of neurons. Its primary function is not known, though it has been implicated as a regulator of synapse formation, neural survival and plasticity, neuritic outgrowth.[9,10]

Aim of the study: This study aim to evaluate the effect of perinatal tramadol treatment of different doses in pregnant mice on thehistological features and on amyloid precursor protein (APP) expression in the cerebral and cerebellar cortices of newborn mice.

MATERIALS AND METHODS

Sixty female pregnant mice were divided into four groups, (15 mice in each group): **The testgroups:** divided into subgroups:(A,B,C) that received different doses of Tramadol

40,60,and 80 mg/kg intra-peritoneally respectively. **The control group,** that received D.W intra-peritoneally. The pregnant female mice treated with tramadol® from day one of pregnancy, after pregnancy confirmation by the presence of vaginal plug. Tramadol obtained from HAVER© 100mg/2ml and diluted in D.W, doses were calculated according to animal weight, and given daily intra-peritoneally. Three doses of Tramadol were selected in the study, (40mg/kg, 60mg/kg, and 80mg/kg), according to[11] who documentedthe best antinoceptive effect of Tramadol in mice based on tail flick test. The newborns mice were euthanized by using chloroform chamber and after death, the head was dissected from the body, skin was removed under the dissecting microscope, and the head wasdivided in sagittal plane into two halves, each half showedthe cerebral and cerebellar cortices. Histological tissue preparation for paraffin blocks according to[12] sections were placed on ordinary slides that stained with H&E for study of histological changes, fisher scientific positive charged slides were used for imunohistochemical staining for anti Anti Ameyeloid precursor protein antibody ab15272 is Rabbit polyclonal to Amyloid precursor protein. Immunohistochemical staining for ab15272, include: Deparafinizing: in xylene for 20 minutes. Rehydration in ethanol, Slides were immersed in PBS(10 minutes), Heat induced antigen retrival was performed by antigen retrival solution (sodium citrate buffer, PH:6), incubation for 5 minutes in autoclave at 120 c°, then cooling down to room temperature for 15 minutes. Slides were washed in PBS, Hydrogen peroxide block drops were added for 10 minutes. Slides were washed in PBS. Protein blockwas added and incubated for 10 minutes at room temperature, Slides were washed in PBS. Primary antibody was diluted at 1/100in PBS, and incubated at room temperature for (2 hours), Slides were washed in BPS, Biotinylated Goat Anti-polyvalentwas added for 20 minutes at room temperature, Slides rinsed in BPS. Streptavidinperoxidase was added, and incubated for 20 minutes at room temperature. Slides were washed in PBS. (30 µl of DAB plus chromogen)was added to 1.5 cc(50 drops) of DAB plus substrate, mixed and applied, incubated for 5 minutes. Slides were rinsed in PBS. Counterstain with Harris Haematoxylin, for 30 seconds and washed in tap water, mounting with water soluble mounting media and cover slips were applied. The immunohistochemical stain assessed by Aperio scope image analysis software positive pixel count (version 9), that used to quantify the amount of a specific stain present in a scanned slide image. The specific color (range of hues and saturation) and three intensity ranges (weak, positive, and strong). For pixels which satisfy the color specification, the algorithm counts the number and intensity sum in each intensity range, the total positivity of the DAP stain was assessed. SSPS version 20 was used for statistical analysis, mean and SD were calculated, ANOVA single factor

analysis used to compare among groups. P value ≤ 0.05 was selected as statistically significant. Un-paired t-test used to compare between groups for the significantp-value.

RESULTS

- **Histological changes in cerebral cortex** were evidently seen in test groups in H&E stained slides, these include: cerebral atrophy, marked reduction in cortex with widening of subarachnoid space (SAS), with loss of characteristic pattern of gyri and sulci of cerebral surface. Figure (1).

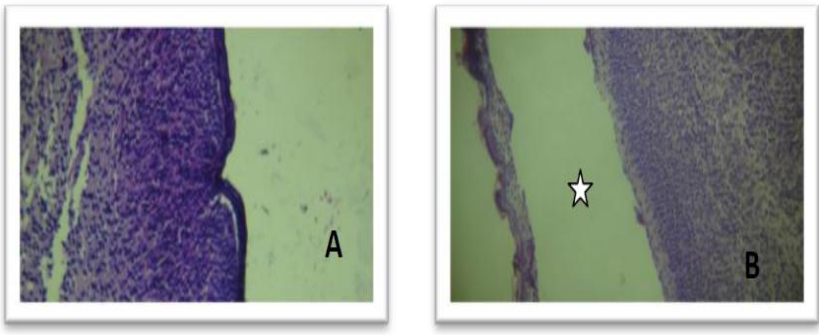

Figure. (1): Atrophy of cerebral cortex, with wide subarachnoid space (star shape),and smooth cerebral surface in test group (B), compared to control with shallow sulci and no atrophic changes (A). H&E 10X.

Apoptotic changes in cells of cerebral cortex these include: cytoplasmic blebbing and vacuolation, nuclear fragmentation, and nuclear pyknosis. (figure2, A,B,C).

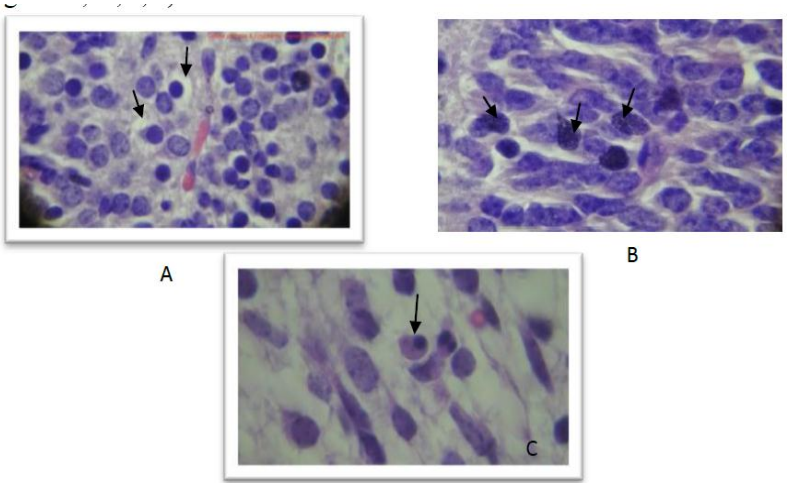

Figure. (2): Apoptotic changes in cerebral cortex of test groups, include: (A) cytoplasmic vacuolation (black arrows) 40X, (B) nuclear fragmentation (black arrows) 40X, (C) nuclear pyknosis (black arrow) 100X. H&E stain.

Vascular changes in cerebral cortical vascular pattern as vascular widening and rupture with presence of areas of haemmorage within the cortex figure 3.

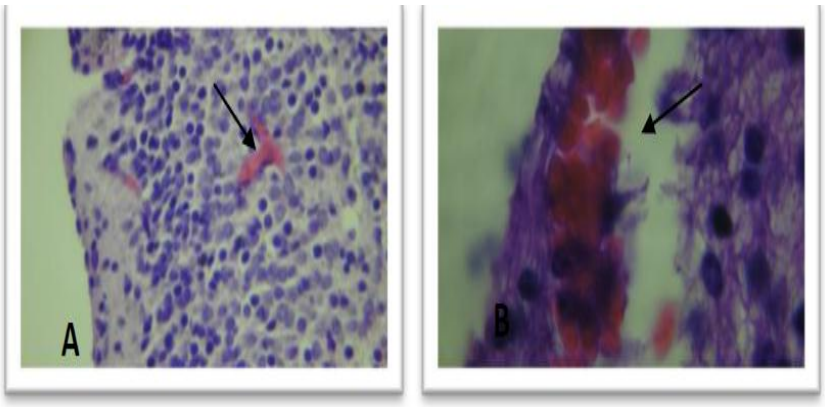

Figure. (3): Vascular changes in cerebral cortex include vascular widening (A) (black arrow) 10X, (B) vascular rupture and privscular oedema (black arrow) 40X, H&E stain.

- **Histological changes in cerebellarcortex**

Slides stained with H&E of cerebellum showed specific changes, these include cerebellar atrophy, with loss of characteristic pattern of folia and sulci (figure 4.A), compared to normal pattern in control group (figure 4.B), absence ofPurkinji cells layer in test group compared to control. (figure 5.A,5.B) Presence of areas of blood cells in the granular layer of the cortex. (Figure 5.B)Dilation of the 4th ventricle and changes in choroid plexus villi arborization (figure 4.B,andfigure.6) compared to control (figure 4.A), and presence of RBC in ventricular cavity of 4th ventricle. (Figure. 6).

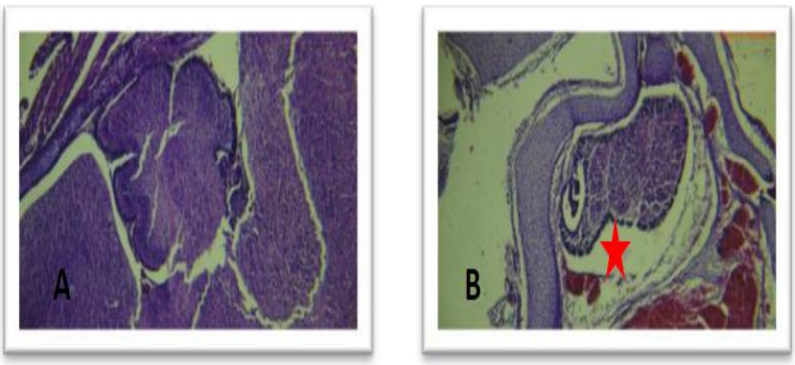

Figure. (4. A) Control cerebellum with normal histological features, (4.B) group A of test groups with cerebella atrophy with ide subarachnoid space (red star), and loss of characterstic pattern of folia and sulci. 10X, H&E stain

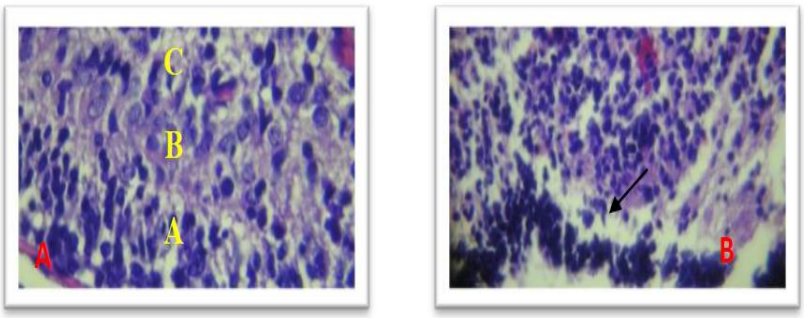

Figure. 5: A Control group with normal cerebellar cortex layers (A. molecular, B. Purkinji cells layer, C. Granular layer),4.B. Group B of test groups showed changes in cerebellar cortex layer with loss of purkinji cells layer (black arrow), 40X, H&E stain.

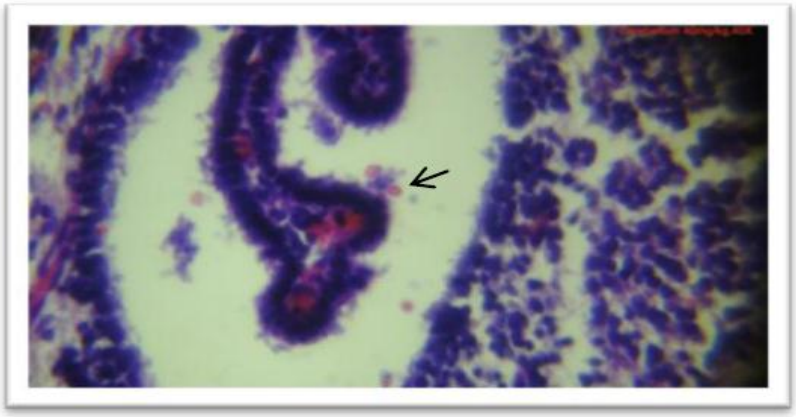

Figure. 6: Choroid plexus of 4th ventricle with change in arborization of villi and presence of RBC in ventricular cavityin group A of test groups (black arrow). 40X, H&E stain

- **Imunohistochemical expression of APPin cerebral cortex**

Assessed by Aperio V9 showed significant changes in APP expression among control and test groups at p≤0.05, that showed an increase in its mean value intest groups with the increase of the tramadol dose of the treatment. Table (1) figure (7), (8), comparison of APP between different test groups and control, that showed significant changes in APP expression at p≤0.05, except between control and group A of test groups. Table (2)

Table. (1): Comparison of Anti Amyloid precursor protein antibody ab15272 in cerebrum by Aperio among control and test groups by ANOVA.

Control Mean±SD	Group A Mean±SD	Group B Mean±SD	Group C Mean±SD	P value
0.004+0.006	0.005+0.003	0.1+0.054	0.393+0.276	< **0.001**

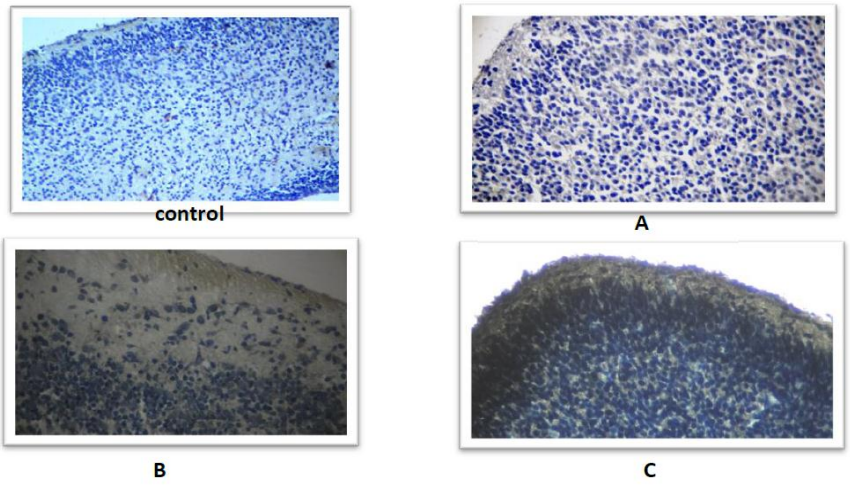

Figure. 7: Variation in the imunohistochemical stain for APP among control group, and test groups (A,B,C) in cerebral cortex, immunohistochemistry for ab15272, 20X.

Table. 2: Comparison of Anti Amyloid precursor protein antibody ab15272 in cerebrum by Aperio between each pair of groups by t-test.

Groups	P value
Control vs group A	0.590
Control vs group B	< **0.001**
Control vs group C	< **0.001**
group A vs group B	< **0.001**
group A vs group C	< **0.001**
group B vs group C	< **0.001**

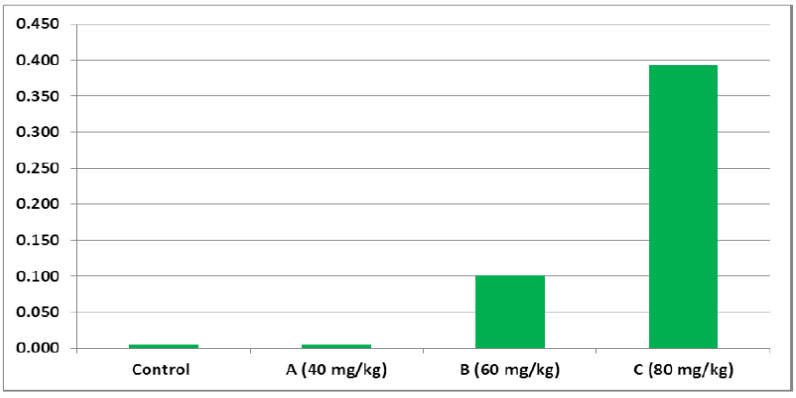

Figure. (8): Comparison of Anti Amyloid precursor protein antibody ab15272 in cerebrum by Aperio among control and test groups.

- **Imunohistochemical expression of APPin cerebellar cortex**

App expression showed a significant changes among control and test groups at p≤0.05 table (3), marked increase in the mean value of APP in test groups was associated with increase of

the dose of the treatment figure 9,10. Significant p-value between groups seen between control and group C of test groups, group A versus group C, and group B versus group C of test groups, table (4).

Table. (3): Comparison of Anti Amyloid precursor protein antibody ab15272 in cerebellum by Aperio among control and 3 study groups by ANOVA.

Control N=30 Mean±SD	Group A N=30 Mean±SD	Group B N=30 Mean±SD	Group C N=30 Mean±SD	P value
0.109+0.193	0.15+0.179	0.199+0.153	0.409+0.199	< **0.001**

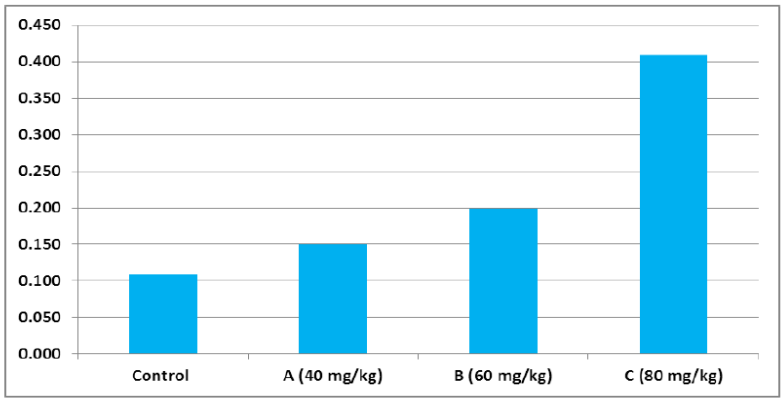

Figure. (9): Comparison of Anti Amyloid precursor protein antibody ab15272 in cerebellum by Aperio among control and 3 study groups.

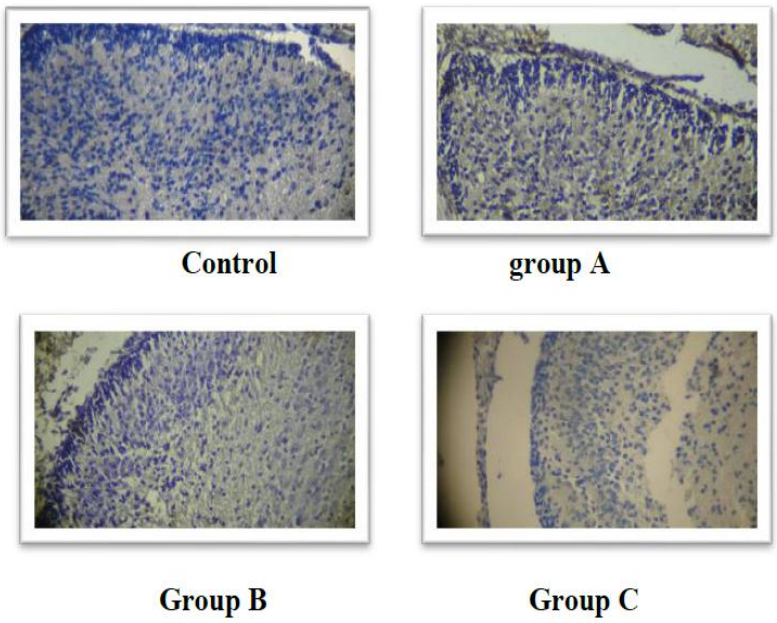

Figure. 10: Variation in the imunohistochemical stain for APP among control group, and test groups (A,B,C) in cerebellar cortex, immunohistochemistry for ab15272,20X.

Table. (4): Comparison of Anti Amyloid precursor protein antibody ab15272 in cerebellum by Aperio between each pair of groups by ttest

Groups	P value
Control vs group A	0.401
Control vs group B	0.052
Control vs group C	< **0.001**
group A vs group B	0.265
group A vs group C	< **0.001**
group B vs group C	< **0.001**

DISCUSSION

This study is designed to demonstrate the effect of the use of tramadol during pregnancy, and its effect on cerebral and cerebellar cortical development and its effect on the expression ofamyloid precursor protein by the neurons of both cortices.

Our results showed marked changes in cerebral and cerebellar corticeshistological features, in form of atrophy that associated with increase in tramadol dose of treatment with widening of subarachnoid space, this attributed to the drug effect on the neural tissue that lead to cell injury upon prolonged use. This agreed with (Liu et al, 2013) who mentioned the chronic effect of such drug on cerebral neurons that induce structural alteration of neurons.[13]

Apoptotic changes seen in cerebral cortex as nuclear fragmentation, cytoplasmic blebbing and vacolation, nuclear pyknosis could related to the drug that injure these cellular structures, so the injured neurons can pass through apoptosis through both intrinsic and extrinsic pathways, this agreed with (Sharifipour et al; 2014) who mentioned apoptosis or programmed cell death is an active process that occurs as a result of the cytotoxic effect of various neurotoxins. In vitro studies, indicated that exposure to opioid receptor agonists increases their liability to death by apoptotic mechanisms.[14]

By light microscopic examination we found that there was marked increase in apoptotic cells proportion, extensive neuronal vacuolization, some regions showed dilated blood vessels. Pyramidal cells loss in cerebral cortex while purkinji cells layer is lost in cerebellar cortex. Apoptotic nuclear changes was also reported by (Zarnescu et al, 2008) in morphine treatment, whofound that the apoptotic cells were present with cytoplasmic contraction, reduction in cell volume and nuclear condensation, and loss of cellular layers. In addition to large amount of apoptotic signals were observed in the nucleus of neurons in the group utilizing opiate as they utilized morphine in their study.[15,16]

Vascular changes seen in our results as dilatation and rupture of vessels ith cortical haemmorage, and changes in the vascular pattern of choroid plexus of 4th ventricle, simiar results were also observed by another study done by (Abou et al, 2014) as they found congestion of sub-meningeal blood vessels and neural degeneration following tramadol treatment.[16]

In addition, (Hassanzadeh et al; 2011) mentionedthat chronic morphine administration in rats is associated with significant changes in the principle proteins involved in the apoptosis signaling which collectively leads to induction of apoptosis.[17]

Induction of apoptosis could be due to oxidative stress effect that exerted by tramadol on nervous tissue, as mentioned by (Popovic et al, 2009)that Tramadol is one of the synthetic opoids that has toxic effects at the cellular level by increasing lipid peroxidation that can be used as a marker of the ROS-induced cell damages.[18]

These data are confirmed by the results from previous studies done by (Zhang et al,2004; Atici et al, 2005) as they demonstrated that treatment with morphine and tramadol yielded an increased malondialdehyde antibody (MDA) level, which suggests an in-creased lipid peroxidation. In addition, they observed a decrease in the level of reduced glutathione in the isolated rat hepatocytes in the case of incubation with different opioids concentration (yielding cell death) and they also, noticed a lowered content of reduced glutathione and activities of catalase, superoxide dismutase and glutathione peroxidase.[19,20]

The oxidative stress induced by tramadol in the brain was reported by (lemarie & Grimm, 2009, Mohamed et al, 2013) as they mentioned the electron transfer chain (ETC) in mitochondria were found to be inhibited by tramadol at high doses, resulted in the generation of reactive oxygen species as a consequence of the intrinsic characteristics of the electron transfer process, and the brain is particularly susceptible to oxidative damage due to its high levels of oxygen consumption, lead to increase levels of polyunsaturated fatty acid and relatively low levels of antioxidants.[21,22] Moreover, oxidative modifications result in a loss of function and lowering of enzyme activity.[23]

These apoptotic changes can be due to tramadol can affect cellular structures as cytoskeleton that may be damaged leading to blebbing of the cytoplasm, as mentioned by (Heidariet al, 2012) as the multiple effects of opioids on neuronal structure (cytoskeleton) that regarded as

the markers of neuronal damage due to long term use of morphine and with Methadone and Buprenorphine opioids (similar in action to tramadol) that presented histological picture of apoptosis.[24]

Atici et al; 2004 observed large amount of apoptotic neurons in the hippocampus of rats treated with opiods, and the expressions of the apoptosis related proteins (Fas, Bcl-2 and caspase-3) presented with alteration in the expressions of Fas and caspase-3 increased markedly and Bcl-2 expression reduced significantly in morphine addiction group and morphine abstinence group. These suggest that long term use of morphine and/or tramadol in increasing doses is found to cause red neuron degeneration and apoptosis in the rat brain, which probably contributes to cerebral atrophy and dysfunction.[25]

Ab15272 that used in this study is Synthetic peptide correspondingto Human Amyloid Precursor Protein aa 44-62 (N terminal)., this comprise the part that a undergoes multiple proteolytic steps, includecleavage by either α- or β-secretase releases the large APP ectodomain, sAPPα and sAPPβ respectively, or called (luminal domain).

Our results showed significant changes in APP among control and test groups, in addition there is a significant increase in APP expression in cerebral and cerebellar cortices that is proportional to the increase in the dose of treatment, and in cerebellar cortex is mainly with controlversus group C, groupA versus C, group B versus C, so in cerebellum its mainly associated with high dose of treatment of 80mg/kg, this could be due toAPP role in protection of neural tissue against insults. This agreed with (Pottier et al., 2012) who reportedevidences from neurological patients and from different disease models hint towards a potential neuroprotective function of APP under conditions of acute cellular insult: as APP is up-regulated following hypoxia, ischemia or traumatic brain injury. This activity- and stress-dependent multi-level relation of APP in neural response strongly suggests a role as an acute phase protein with functions in cellular survival under metabolically challenging conditions.[26]

APP alsoinvolved in regulation of synaptic vesicle exocytosis[27] glutamatergic, GABAergic and cholinergic synaptic transmission[28, 29, 30] and synapse formation.[31] Interestingly, it also regulates endosomal phosphoinositide metabolism and prevents neurodegeneration[32], and it interacts with a large variety of survival-related cascades.[33, 34]

While over-expression of APP following mechanical insults has been observed several decades ago, the functional effects remained unclear until recently. Evidence from different animal models points towards an acute neuroprotective effects of APP and APPsa in traumatic brain injury. In diffuse traumatic injury in rats, intra ventricular administration of after the insult reduced axonal injury and apoptosis and improved motor and cognitive outcome.[35] It was reported that Posttraumatic application of exogenous APPsa limited these deficits.[36]Additional study showed that, the neuroprotective properties when intra ventricularly applied post-trauma, was enough to significantly improve histological and functional outcome.[37]

The raise in APP expression by cerebral cortex that is proportional to the increase in the dose of tramadol treatment is related to its protective functions to the developing cerebral and cerebellar cortices neural tissue. This can be related to that APP mediates many effects on brain development and supports several cognitive functions. In addition, has been shown to mediate a variety of neuroprotective and trophic effects.[38,39, 40]

APPsb fails to mimic the beneficial effects of APPsa, although there is only a difference of 16aminoacids between both proteins.[38] In other studies, mentioned that, the trophic effects ofAPPsb were detected, still less potent than those of APPsa.[41] Interestingly, APPsb was found to undergo further proteolytic cleavage and bindto‘‘deathreceptor6’’, activatingcaspase-6 and thus contributing to neurodegeneration.[42]

Tramadol brain tissue injury could attributed to hypoxia and haemmorage as the drug affect cerebral cortical vessels as shown in our results, thatlead to over-expression of APP. It was found evidence from different animal models points towards an acute neuroprotectiveeffect of APPand APPsa in TBI (traumatic brain injury).[35] More over in diffuse traumatic injury in rats, intra-ventricular administration of APPsa 30 minutesafter the insult reduced axonal injury and apoptosis and improved motor and cognitive outcome.[43]

APP’s known trophic effects on neuronal viability, cell adhesion, axonogenesis, dendritic arborization and dendritic spines may also contribute to recovery from traumatic and metabolic insults and counter act degeneration.[41] All these findings of cerebral injury, apoptotic changes and over expression of APP by cerebral cortex neurons points toward the harmful effects of tramadol use during pregnancy specifically if its use starts from early pregnancy and extends over long periods.

REFRENCES

1. Raffa RB, Friderichs E. The basic science aspect of tramadol hydrochloride. Pain Rev., 1996; 3: 249-71.
2. Katz WA "Pharmacology and clinical experience with tramadol in osteoarthritis". Drugs, 1996; 52(3): 39–47. doi:10.2165/00003495-199600523-00007. PMID 8911798.
3. Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, Vaught JL. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an 'atypical' opioid analgesic. J Pharmacol Exp Ther, 1992; 260(1): 275-85.
4. Granados-Soto V and Arguelles CF. Synergic antinociceptive interaction between tramadol and gabapentin after local, spinal and systemic administration. Pharm, 2005; 74: 200-208.
5. Shipton EA. Tramadol--present and future. Anaesth Intensive Care, 2000; 28: 363-374.
6. Gillen C., Haurand M., Kobelt D. J., Wnendt S., NS. Arch. Pharmacol., 2000; 362: 116-121.
7. Oliva P., Aurilio C., Massimo F., Grella A., Maione S., Grella E., Scafuro M., Rossi F., Berrino L., Eur. J. Pharmacol., 2002; 445: 179-185.
8. Bloor M, Paech MJ, Kaye R. "Tramadol in pregnancy and lactation". International Journal of Obstetric Anesthesia, 2012; 21(2): 163-67. doi:10.1016/j.ijoa.2011.10.008. PMID 22317891.
9. Turner PR, O'Connor K, Tate WP, Abraham WC (May 2003). "Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory". Progress in Neurobiology. 70 (1): 1–32. doi:10.1016/S0301-0082(03)00089-3. PMID 12927332.
10. De Strooper B, Annaert W. Proteolytic processing and cell biological functions of the amyloid precursor protein. J Cell Sci. 2000; 113(Pt 11):1857–1870.[PubMed: 10806097]
11. Fariborz Keyhanfar, Manzumeh Shamsi Meymandi, Gholamreza Sepehri; et al.Evaluation of Antinociceptive Effect of Pregabalin in Mice and its Combination with Tramadol using Tail Flick Test. Iranian Journal of Pharmaceutical Research, 2013; 12(3): 483-493.
12. Bancroft & Stevens 1987 Survana, K.S & Bancroft, J.D. (2013) Bancroft´s theory and practice of histological techniques.7th edition.
13. Liu LW, Lu J, Wang XH, Fu SK, Li Q, Lin FQ. Neuronal apoptosis in morphine addiction and its molecular mechanism. Int J Clin Exp Med., 2013; 6: 540-5.
14. Sharifipour M, Izadpanah E, Nikkhoo B, Zare S, Abdolmaleki A, Hassanzadeh K, Moradi F, Hassanzadeh K. A new pharmacological role for donepezil: attenuation of morphine-induced tolerance and apoptosis in rat central nervous system. J Biomed Sci., 2014; 23: 21-6.
15. Zarnescu O, Brehar FM, Chivu M, Ciurea AV. Immunohistochemical localization of caspase-3, caspase-9 and Bax in U87 glioblastoma xenografts. J Mol Histol, 2008; 39: 561-569.

16. Abou El Fatoh MF, Farag M, Sayed AE, Kamel MA, Abdel-Hamid N, Hussein M, Salem GA. Some Biochemical, Neurochemical, Pharmacotoxicological and Histopathological Alterations Induced by Long-term Administration of Tramadol in Male Rats. Int J Pharm Sci., 2014; 4: 565-571.
17. Hassanzadeh K, Habibi-Asl B, Farajnia S, Roshangar L. Minocycline prevents morphine-induced apoptosis in rat cerebral cortex and lumbar spinal cord: a possible mechanism for attenuating morphine tolerance. Neurotox Res., 2011; 21: 649-659.
18. Popovic M, Janicijevic-Hudomal S, Kaurinovic B, Rasic J, Trivic S, Vojnović M. Antioxidant effects of some drugs on immobilization stress combined with cold restraint stress. Molecules, 2009; 14: 4505-16.
19. Zhang YT, Zheng QS, Pan J, Zheng RL. Oxidative Damage of Biomolecules in Mouse Liver Induced by Morphine and Protected by Antioxidants. Basic Clin Pharmacol Toxicol, 2004; 95: 53-58.
20. Atici S, Cinel I, Cinel L, Doruk N, Eskandari G, Oral U. Liver and Kidney Toxicity in Chronic Use of Opioids: An Experimental Long Term Treatment Model. J Biosci, 2005; 30: 245-252.
21. Lemarie A, Grimm S. Mutations in the heme b-binding residue of SDHC inhibit assembly of respiratory chain complex II in mammalian cells. Mitochondrion, 2009; 9: 254-260.
22. Mohamed TM, Abdel Ghaffar HM, El Husseiny RM. Effects of tramadol, clonazepam, and their combination on brain mitochondrial complexes. Toxicol Ind Health 2013;[Epub ahead of print].
23. Butterfield DA, Reed T, Newman SF, Sultana R. Roles of amyloid beta-peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive impairment. Free Radic Biol Med., 2007; 43: 658-77.
24. Heidari Z, Mahmoudzadeh-Sagheb H, Kohan F. A Quantitative and Qualitative Study of Rat Testis Following Administration of Methadone and Buprenorphine. International Journal of High Risk Behaviors and Addiction, 2012; 1: 14-17.
25. Atici S, Cinel L, Cinel I, Doruk N, Aktekin M, Akca A, Camdeviren H, Oral U. Opioid neurotoxicity: comparison of morphine and tramadol in an experimental rat model. Int J Neurosci, 2004; 114: 1001-11.
26. Pottier, C., Wallon, D., Lecrux, A.R., Maltete, D., Bombois, S., Jurici, S., etal. Amyloid-b protein precursor gene expression in Alzheimer's disease and otherconditions. J. Alzheimers Dis. 2012; 28: 561–566. doi: 10.3233/JAD-2011- 111148.
27. Kohli, B.M., Pflieger, D., Mueller, L.N., Carbonetti, G., Aebersold, R., Nitsch, R.M., etal. Interactome of The Amyloid Precursor Protein APP in brain reveals aprotein network

involved insynapticvesicleturnover and a close association with Synaptotagmin-1. J. Proteome Res., 2012; 11: 4075–4090. doi: 10.1021/pr300123.

28. Wang, P., Yang, G., Mosier, D.R., Chang, P., Zaidi, T., Gong,Y., etal. (2005). Defective neuromuscular synapses in mice lacking amyloid precursor protein (APP) and APP-likeprotein2. J. Neurosci. 25, 1219–1225. doi: 10.1523/JNEUROSCI.4660-04.2005.
29. Schrenk-Siemens, K.,Perez-Alcala, S.,Richter, J.,Lacroix,E., Rahuel, J., Korte, M., et al. (2008). Embryonicstemcell-derived neurons as a cellular system to study gene function: lack of amyloid precursor proteins APPand APLP2 leads to defective synaptic transmission Stem Cells 26, 2153–2163. doi: 10.1634/stemcells.2008-0010.
30. Priller, C., Bauer, T., Mitteregger, G., Krebs, B., Kretzschmar, H.A., and Herms, J. (2006). Synapse formation and functionis modulated by the amyloid precursor protein. J. Neurosci. 26, 7212–7221. doi: 10.1523/JNEUROSCI.1450- 06.2006.
31. Russo, C., Venezia, V., Repetto, E., Nizzari, M., Violani, E., Carlo, P., etal. The amyloid precursor protein and its network of interactingproteins: physiological and pathological implications. Brain Res. Rev., 2005; 48: 257–264. doi: 10.1016/j.brainresrev.2004.12.016.
32. Venezia, V., Nizzari, M., Carlo, P., Corsaro, A., Florio,T., and Russo, C. Amyloid precursor protein and presenilin involve mentincellsignaling. Neurodegener. Dis., 2007; 4: 101–111. doi: 10.1159/000101834.
33. Plummer, S., VandenHeuvel, C., Thornton, E., Corrigan, F., and Cappai, R. The neuroprotective properties of the amyloid precursorprotein following traumaticbraininjury. Aging Dis., 2016; 7: 163–179. doi: 10.14336/AD. 2015.0907.
34. Corrigan, F.,Thornton,E.,Roisman,L.C.,Leonard,A.V.,Vink,R., Blumbergs, P.C.,etal. Theneuroprotectiveactivityoftheamyloid precursor proteinagain sttraumaticbraininjuryismediatedviatheheparin binding siteinresidues96–110. J. Neurochem, 2014; 128: 196–204. doi: 10.1111/jnc. 12391
35. Hick, M., Herrmann,U.,Weyer,S.W.,Mallm,J.-P.,Tschäpe,J.-A., Borgers, M.,etal. Acutefunctionofsecretedamyloidprecursor protein fragmentAPPsa in synapticplasticity. Acta Neuropathol, 2015; 129: 21–37. doi: 10.1007/s00401-014-1368-x
36. Fol, R.,Braudeau,J.,Ludewig,S.,Abel,T.,Weyer,S.,Roederer,J.-P.,etal.(2016). Viral genetransferofAPPsa rescues synapticfailureinanAlzheimer'sdisease mouse model. Acta Neuropathol. 131, 247–266. doi: 10.1007/s00401-015- 1498-9
37. Hefter, D., Kaiser, M.,Weyer,S.W., Papageorgiou,I.E., Both,M.,Kann,O., et al.(2016). Amyloid precursor protein protectsneuronalnetworkfunction after hypoxiaviacontrolofvoltage-gatedcalciumchannels. J. Neurosci. 36, 8356–8371. doi: 10.1523/JNEUROSCI.4130-15.2016

38. Chasseigneaux, S., Dinc, L., Rose,C., Chabret, C., Coulpier, F., Topilko, P., etal. (2011). Secreted amyloid precursor protein b and secreted amyloid precursor protein alpha induce axon outgrowth in vitro through Egr1signalingpathway. PLoS One 6:e16301. doi: 10.1371/journal.pone.0016301
39. Nikolaev, A., McLaughlin, T.,O'Leary, D., and Tessier-Lavigne, M. N-APP binds DR6tocause axon pruning andneurondeathviadistinctcaspases. Nature, 2009; 457: 981-989. doi: 10.1038/nature07767
40. Thornton, E., Vink, R., Blumbergs, P.C., and Van Den Heuvel, C. Soluble amyloid precursorprotein a reduces neuronal injury and improves functional outcome following diffuse traumaticbraininjuryinrats. Brain Res., 2006; 1094: 38–46. doi: 10.1016/j.brainres.2006.03.107.
41. Lee, K.J., Moussa, C.E.H., Lee, Y., Sung, Y., Howell, B.W., Turner, R.S., et al. Betaamyloid-in dependent role of amyloid precursorproteinin generation and maintenance of dendritic spines. Neuroscience, 2010; 169: 344–356. doi: 10.1016/j.neuroscience.2010.04.078.
42. Balklava, Z., Niehage, C., Currinn, H., Mellor, L.,Guscott, B., Poulin, G., etal. (2015). the amyloid precursor proteincontrols PIK fyvefunction. PLoS One 10:e0130485. doi: 10.1371/journal.pone.0130485.
43. Thornton, E., Vink, R., Blumbergs, P.C., and Van Den Heuvel, C. (2006). Soluble amyloid precursorprotein a reduces neuronal injury and improves functional outcome following diffuse traumatic brain injury in rats. Brain Res. 1094, 38–46. doi: 10.1016/j.brainres.2006.03.107.