ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

MEDICINAL CHEMISTRY

# CHRONIC SIMULTANEOUS ADMINISTRATION OF ACETAMINOPHEN AND ETHANOL DEPLETES PURKINJE CELLS IN THE (VERMIS) CEREBELLAR CORTEX OF ADULT WISTAR RATS (*RATTUS NORVEGICUS*)

**FAKUNLE P.B*, AJIBADE A.J AND OYEWO E.B**

**Anatomy Department, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.**
**Anatomy Department, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.**
**Biochemistry Department, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.**

**FAKUNLE P.B**
**Anatomy Department, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.**

***Corresponding author**

## ABSTRACT

Acetaminophen, also known as paracetamol, is a drug with potent antipyretic and analgesic actions but with very weak anti-inflammatory activity while ethanol is a depressant and regarded as a social drug. However very scanty literature is available especially when these drugs are consumed chronically and simultaneously despite their high degrees of abuse and misuse on the purkinje cells in the vermis of the cerebellum. In this study Forty adult wistar rats of average weight 150±20.2g were randomly distributed into four groups of treatments $T_1$,$T_2$$T_3$ and control C (N=10) . The animals were fed on standard laboratory mouse chow with water provided *ad libitum***.** For a period of six weeks, animals in group $T_1$ received 100mg/Kg.bwt. acetaminophen and 25% ethanol in 2% sucrose solution while group $T_2$ animals received 25% ethanol in 2% sucrose solution. $T_3$ animals were given 100mg/Kg.bwt. acetaminophen and group C animals were given only distilled water. The animals were sacrificed by cervical dislocation and the cerebellar cortex were dissected out and processed for routine histological techniques. Shrunken brains were seen in the treatment groups $T_1$ and $T_2$ with significant brain weight loss ($P<0.01$) compared to the control. Significantly reduced purkinje cells neuronal density ($P<0.05$) of 49% and 43% neuronal loss respectively in treatment groups $T_1$ and $T_2$ compared to control group was recorded. The purkinje cells reduction observed as well as brain weight loss may adversely affect the normal functions of the cerebellum being a comparator and coordinator.

ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011

## KEYWORDS

Acetaminophen, Ethanol, Cerebellum, Purkinje cells, Adult brain, Neuronal density

## INTRODUCTION

Acetaminophen (Paracetamol) is a widely-used analgesic and antipyretic medication. It is commonly used for the relief of fever, headaches, and other minor aches and pains, and is a major ingredient in numerous cold and flu remedies[1]. In combination with non-steroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics, paracetamol is used also in the management of more severe pain (such as postoperative pain)[1].However excessive use of paracetamol can damage multiple organs, especially the liver and kidney. In both organs, toxicity from paracetamol is not from the drug itself but from one of its metabolites as *N*-acetyl-*p*-benzoquinoneimine (NAPQI)[2] (Mohandas et al., 1981).Ethanol is psychoactive drug which also play very active role as a central nervous system depressant[3] but inability to modify drinking action which has always culminated into chronic consumption is a problem of public health concern[4]. Both acetaminophen and ethanol have been reported to readily cross the blood brain barrier[5].The cerebellar purkinje cells constitute the principal neurons of the cerebellum[6].They are highly differentiated neurons whose cell bodies form a monolayer between molecular and granular layers of cerebellar cortex[7].Heavy duty workers especially over the coast of Africa and other parts of the developing and even in developed world are involved in both conscious and unconscious chronic abuse and misuse of acetaminophen as a pain reliever and ethanol being a central nervous system depressant. High doses of both acetaminophen and ethanol have traditionally been associated with neurodegenerative disorders due to implications of oxidative stress[8] in the brain. Because of the crucial roles acetaminophen and ethanol played in the daily life of both healthy and unhealthy individual, this study focuses on the integrity of the purkinje cells when chronically assaulted by acetaminophen and ethanol.

## MATERIALS AND METHODS

Forty adult presumably, healthy wistar rats of both sexes, average weight 200±3.23g were maintained under standard laboratory conditions for an acclimatization period of 2 weeks in the Animal Holdings of Anatomy Department, Ladoke Akintola University of Technology Ogbomoso. The rats were fed with standard laboratory mouse chow (Ladokun feeds, Ibadan) and were given water *ad libitum*. Daily weights were taken and documented.

After acclimatization, the rats were randomly assigned into four groups (N=10) such that $T_1$, $T_2$ and $T_3$ served as treatment groups, while C served as the control group. $T_1$ received 100mg/kg body weight acetaminophen and 2% sucrose in 25% ethanol solution as their drinking water, $T_2$ took neutral 2% sucrose in 25% ethanol solution as their drinking water while $T_3$ received 100mg/kg body weight of acetaminophen in distilled water. The 2% sucrose in 25% ethanol solution were replaced afresh daily at 18.00 hours G.M.T. The rats in group C received distilled water *ad libitum*. All the animals were exposed for a period of 6 weeks.

Absolute ethanol was obtained from Sigma Laboratory Ltd, San Francisco, U. S. A. while acetaminophen was obtained from Emzor Phamaceutical industrial limited, Nigeria.

At the end of administration, all the rats were sacrificed by cervical dislocation and kept in 10% formol calcium fixative for 72hours. Regions cerebellum were then excised out and processed for routine histological techniques sectioned at 6µwith a Rotary Microtome and stained using Cresyl violet method as described by[9] Venero et al 2000 for nissl's substance. Qualitative observations of cerebellar sections were done with every 10th section chosen from each animal. Using brightfield compound Nikon microscope, YS100 (attached with Nikon camera), the slides were examined and photographed under 400X objective. Using Image-Pro Express software, counts of neurons with prominent nucleolus within a measured monolayer of purkinje cells at several microscopic fields were done. The neuronal transverse diameter for the purkinje cells was also determined. The absolute neuronal density per unit area of section for each region was estimated as described by[10]

***Statistical analysis**:*

The data were analyzed using the computerized statistical package 'SPSS Version 11'. Mean and standard error of mean (SEM) values for each experimental group was determined. The means were compared by analysis of variance at a level of significance of 95% and 99%. Independent samples t-test was performed on the counts of each area of purkinje cells layer to determine if there is any statistically significant difference in absolute neuronal count between the control and treatment groups.

## RESULTS

The brain weight in groups T1 and T2 as recorded showed statistically significant **P<0.01** of Mean±SEM (1.58 ± 0.11 and 1.60 ± 0.02)g respectively when compared to the control group C with Mean±SEM (1.82 ± 0.13)g in **Table 1**.

**TABLE 1**

***Mean± SEM of wet brain weight of rats at the end of administration.***

| Group | N | Mean± SEM (g) | F-value | D.O.F | 2-Tprob. |
|---|---|---|---|---|---|
| C | 10 | 1.82 ± 0.21 | 3.41 | 5 | 0.002 |
| T1 | 10 | 1.58± 0.20 | | | |
| T2 | 10 | 1.60± 0.21 | | | |
| T3 | 10 | 1.79± 0.21 | | | |

**P< 0.01**

The mean brain weight loss was insignificant (**P>0.01**) for group T3 of Mean±SEM(1.79 ± 0.21)g as seen in **Table 1** when compared to the control group. The results obtained from **Table 2** clearly showed that treatment groups T1 and T2 had significantly (**P<0.05**) reduced neuronal density for the purkinje cells Mean±SEM (728.16±12.16 and 806±16.11)/sq.cm when compared to the control group with Mean±SEM (1426.11±8.13)/sq.cm which translates to a percentage neuronal loss of 49% and 43% for treatment groups T1 and T2.

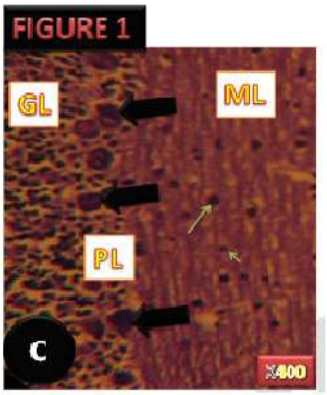
**TABLE 2**
***Mean ± SEM (Neuronal density and cells diameter) of Purkinje cells***

| Group | Purkinje cells /sq.cm | Neuronal loss(%) | Purkinje cells diameter (µm) |
|---|---|---|---|
| C | 1426.11±8.13 | - | 1.30±0.01 |
| T1 | 728.16±12.16* | 49 | 0.66±0.02* |
| T2 | 806±16.11* | 43 | 0.70±0.02* |
| T3 | 1268.01±10.18 | 11 | 1.20±0.01 |

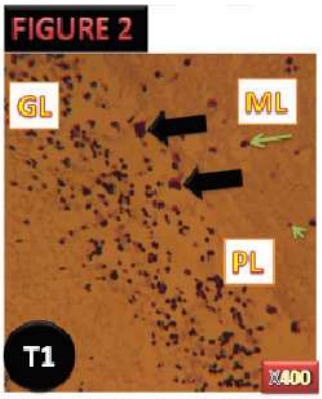
* Significant difference **P<0.05** when compared to the control

Although 11% neuronal loss was recorded for treatment group T3 with Mean±SEM (1268.01±10.18)/sq.cm of purkinje cells, however this was statistically insignificant (**P>0.05**) when compared to the control group as seen in **Table 2**. The neuronal transverse diameter for the purkinje cells revealed statistically significantly reduced values **(P<0.05**) for treatment groups T1and T2 of Mean±SEM (0.66±0.02 and 0.70±0.02)µm in **Table 2** compared to Mean±SEM (1.30±0.01)µm obtained from the control section. The values obtained for neuronal transverse diameter for the purkinje cells in treatment group T3 was Mean±SEM (1.20±0.01)µm which is insignificant **P>0.05** as compared to the control group as seen in **Table 2.**

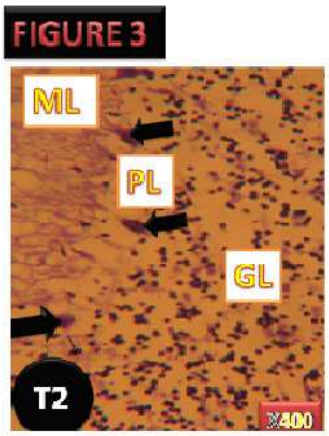
The control group (C) is normal in its histological appearance as shown in **Figure 1**. All the layers of cerebellum from Purkinje layer (PL), Molecular layer (ML) to the Grannular layer (GL) including the White matter (WM) revealed prominent well represented cytoarchitectural clearity. However, histological findings showed in the purkinje layer, reduced and distorted Purkinje cells with some broken axons while the granular layer appeared very scanty with sparce distribution of the glial cells in the molecular layer in treatment groups ($T_1$) and ($T_2$) (**Figures 2 and 3**) although the findings in treatment group ($T_2$) was not as pronounced as in treatment group ($T_1$). Histological findings in treatment group ($T_3$) (**Figure 4**) showed onset of neuronal damage in the granular layer, prominent Purkunje cells and prominent neuron and glia cell in the molecular layer.



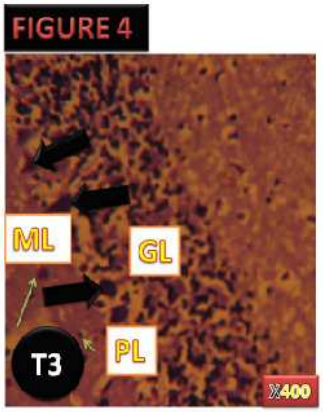

Photomicrograph of Cerebellum ( control section C ) showing distinct layers of ML(Molecular layer), PL(Purkinje layer) and GL(Grannular layer). Note the normal purkinje cells (black arrows) the evenly distributed grannular cells with the normal glia cells (Green arrow and arrow-head) Nissl stain x400.




Photomicrograph of Cerebellum (Treatment section T1) showing in the purkinje layer(PL), reduced and distorted Purkinje cells (black arrows ) with some broken axons while the cells in the granular layer (GL) appeared very scanty with sparce distribution of the glial cells (Green arrow and arrow-head) in the molecular layer(ML) Nissl stain x400.




Photomicrograph of Cerebellum (Treatment section T2) showing distorted Purkinje neurons (black arrows) in the purkinje layer(PL), less populated granular cells of the granular layer(GL) and few glial cells (Green arrow and arrow-head) in the molecular layer(ML) Nissl stain x400




Photomicrograph of Cerebellum (Treatment section T3) showing prominent Purkinje neuron with few distortion ( black arrows) in the purkinje layer (PL) , evenly distributed grannular cells in the grannular layer(GL) and glia cells (Green arrow and arrow-head) in the molecular layer (ML) Nissl stain x400.

## DISCUSSION

The results of this study point to the fact that chronic simultaneous administration of acetaminophen and ethanol had adverse effects on the brain weight and purkinje cell population of the cerebellum. The shrunken brains reported in this study are in conformation with earlier findings of[11] Fakunle et al, 2011 where chronic intake of ethanol accounted for brain weight loss. The weight loss recorded in the wet brains of treatment groups T1 and T2 (Mean±SEM (1.58 ± 0.11 and 1.60 ± 0.02g)) respectively when compared to the control group C with Mean±SEM (1.82 ± 0.13)g can be due to effects of acetaminophen and ethanol as findings have revealed that alcohol impairs nutrient absorption. Alcohol also inhibits the breakdown of nutrients into usable substances, by decreasing the secretion of digestive enzymes from the pancreas[12].There have been many reports claiming that the hepatotoxicity of paracetamol (acetaminophen) is increased in chronic alcoholics, and that such individuals not only carry an increased risk of severe and fatal liver damage after acute overdosage but that similar serious liver damage may also occur with 'therapeutic' use[13].Structural brain damage including shrinkage has also been credited to effects of chronic consumption of ethanol[14] .In

this study the weight loss obtained in the group T3 is less marked when compared to the control group but still more significant when compared to treatment groups T1 and T2 from table I and this further underlines the fact that chronic simultaneous consumption of acetaminophen and ethanol is of immense adverse effects. A change in the dimensions of a cell has been reported[15]to have potentials of reflecting the internal changes in the ultrastructure of the cell, hence the transverse diameter of a purkinje cell could be used as a representative index of its size. Overdose of acetaminophen, a widely used analgesic drug, can result in severe hepatotoxicity and is often fatal. This toxic reaction is associated with metabolic activation by the P450 system to form a quinoneimine metabolite, N-acetyl-p-benzoquinoneimine (NAPQI), which covalently binds to proteins and other macromolecules to cause cellular damage[16]. A possible mechanism for alcohol-induced brain damage involves oxidative stress of neurons which as a by-product of alcohol metabolism, free radicals may be formed. These are highly reactive molecular fragments that are capable of inflicting serious damage on cells if they are not quickly neutralized[17]. Normally, free radicals are rapidly inactivated by antioxidants, which are protective molecules that inhibit oxidation. However, if these defenses are impaired, or if there is an overproduction of free radicals, the result is oxidative stress. This imbalance between increased production of free radicals and decreased availability of antioxidants can result in cell death. Cell degeneration has been classified mainly into two types as necrosis and apoptosis. According to[18], necrosis affects cell population and is often characterized by inflammation and release of intracellular organelles as a result of disruptions of the plasma membrane as well as cytoplasmic swelling. Apoptosis is a genetically determined, biologically meaningful, active process playing a role opposite to mitosis in tissue size regulation, shaping organs and removing cells that are immunologically reactive against self, infected or genetically damaged whose continuous existence pose a danger to the host. It is actively involved in physiological and developmental processes[19]. In this study, the mean diameters of the purkinje cells revealed significantly reduced values **(P<0.05**) for treatment groups T1and T2 of Mean±SEM (0.66±0.02 and 0.70±0.02)µm compared to Mean±SEM (1.30±0.01)µm obtained from the control group and values obtained for neuronal transverse diameter for the purkinje cells in treatment group T3 was Mean±SEM (1.20±0.01)µm which is insignificant **P>0.05** as compared to the control group. From these above data the observed cellular degeneration which is clearly evident in the microarchitectural picture seen if **Figures 2** and **3** as compared to **Figures 1** and **4** is apoptotic in nature confirming the earlier reports that ethanol triggers widespread apopototic neurodegeneration in the brain[20] just as acetaminophen-induced neuronal death exhibits the hallmarks of apoptotic death. Hence it appeared that neuronal degeneration is more marked when both acetaminophen and ethanol are taken chronically and simultaneously. According to[21], the purkinje cells are among the neurons of the brain that have been reported to be sensitive to ischaemia, ethanol bilirubin and biphenyl hydration. This category of substances may trigger an apoptotic pathway within the brain although can be prevented by manipulating the control at molecular levels. Purkinje cells population from findings of this study showed significant P<0.05 decrease of about 49% and 43% neuronal loss in treatment group T1 andT2 respectively compared to the control and an insignificant value of 11% loss in the treatment group T2 and this underscores fact that when ethanol and acetaminophen are chronically combined the aftermath effect is more deleterious than when either drug is used singly. Neuronal counting from histological sections

confirmed the morphological observations. The reduced population of purkinje cells observed here may underline adverse effects of chronic simultaneous intake of acetaminophen and ethanol resulting in impairment of the functions of purkinje cells being the principal neurons of the cerebellum which provide the sole output from cerebellar cortex while all other neurons termed intrincic neurons. Hence it is concluded that the principal functions of cerebellum are compromised following long term simultaneous intake of acetaminophen and ethanol.

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