



Full Length Research Paper

A study on microanatomical integrity of neuronal cells in dorsal lateral geniculate nucleus of adult wistar rats (*Rattus norvegicus*) following chronic simultaneous administration of ethanol and acetaminophen

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Abstract

Control of drug use is of paramount importance as regards the public health concern and among the leading most abused drugs are ethanol and acetaminophen while the maintenance of uninterrupted visual signals is of crucial importance to an individual survival of which Lateral geniculate body roles as a vital relay station. In this study, long term effects of simultaneous administration of ethanol and acetaminophen were investigated on the lateral geniculate body. Forty adult wistar rats of both sexes of average weight $185 \pm 20.2g$ were randomly distributed into four groups of treatments T1, T2, T3 and control C (N=10). The animals were fed on standard laboratory mouse chow with water provided *ad libitum*. For a period of 6 weeks, animals in group T1 received 100mg/Kg.bwt. acetaminophen and 25% ethanol in 2% sucrose solution while group T2 animals received 25% ethanol in 2% sucrose solution. T3 animals were given 100mg/Kg.bwt. acetaminophen and group C animals were given only distilled water. At the end of administration all the animals were sacrificed by cervical dislocation and the LGB were dissected out and processed for routine histological techniques and bioassay of succinic dehydrogenase. Shrunken brains were seen only in the treatment groups T1 and T2 with significant brain weight loss ($P < 0.01$) compared to the control while T3 brain were insignificantly reduced compared to the control. Histological findings revealed scantily distributed pyknotic neuronal cells in the T1 and T2 groups while T3 sections appeared normal with few distortions compared to normal pictures obtained in the control group. Significantly ($P < 0.05$) reduced neuronal cells density of 46% and 39% neuronal loss respectively was obtained in treatment groups T1 and T2 respectively compared to control group as against an insignificant 9% loss seen control group. However, the enzymatic of SDH was markedly reduced in T1 and T2 as compared to high activities recorded in the control and T3 groups. These neuronal alterations may underline visual defects associated with lateral geniculate body as a visual relay station following chronic simultaneous consumption of ethanol and acetaminophen.

Keywords: Neurons, Ethanol, acetaminophen, succinate dehydrogenase, vision.

INTRODUCTION

Drug users abuse over-the-counter products and prescription medicines when they are unable to obtain their usual illicit street drugs. Abuse of drug can be described as the use of medication "recreationally," in

larger amounts than prescribed, in greater frequency, for different indications or by different routes, usually resulting in adverse consequences (Abbott and Fraser, 1998).

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 (Roberts and Marrow, 2001). In combi-

nation 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). 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) (Mohandas et al., 1981). Ethanol is psychoactive drug which also play very active role as a central nervous system depressant but inability to modify drinking action which has always culminated into chronic consumption is a problem of public health concern (Fakunle, 2006) and a major characteristic feature of alcohol use disorders is the consumption of dangerous amounts of alcohol despite the knowledge that problems occur during drinking (Fulton and Kim, 2008). The most prevalent ethanol – associated brain impairments affect visuospatial abilities and higher cognitive functioning (Oscar Berman, 2000). In the light of this, since visual signals are transmitted through the visual pathway of which lateral geniculate body which is an intracranial visual relay station, it is significant to study the effects of ethanol and acetaminophen on the integrity of this sub-cortical structure.

MATERIALS AND METHODS

Forty adult healthy wistar rats of both sexes of average weight 210 ± 4.22 g 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. During which they were fed with standard laboratory mouse chow (Ladokun feeds, Ibadan) and provided water *ad libitum*. Daily weights were taken and documented. At the end of 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 2% sucrose in 25% ethanol solution as their drinking water while T_3 received 100mg/kg body weight of acetaminophen and distilled water. The rats in group C received distilled water. The 2% sucrose in 25% ethanol solutions were replaced afresh daily at 18.00 hours G.M.T. All the animals were exposed for a period of 6 weeks. Changes in body weights and volume of ethanol consumed were documented. Bottles containing absolute ethanol were obtained from Sigma Laboratory Ltd, San Francisco, U. S. A. while acetaminophen was obtained from Emzor Pharmaceutical industrial limited, Nigeria. At the end of administration, all the rats were sacrificed by cervical dislocation and each wet brain specimens were weighed and then fixed in 10% formol calcium fixative for 72hours

after which regions of Lateral geniculate body (LGB) were then dissected out using Paxinos stereotaxic coordinate method (Paxinos and Waston, 1998). The brain specimens were processed for routine histological techniques and sectioned at 6μ following which they were stained with Cresyl violet as described by Venero et al 2000 for nissl's substance. Qualitative observations of areas of lateral geniculate body were done on 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. For each slide, areas of LGB were randomly selected. Using Image-Pro Express software, count of neurons with prominent nucleolus within a measured rectangular area was performed in the selected regions. Random measurements of neuronal cell diameter were also taken for each region. The absolute neuronal density (P) per unit area of section was estimated using the formula $P = A \cdot M / L + M$ [Abercrombie, 1946]; M = Section thickness in micron (6 micron); L = Mean nuclear diameter of respective area; A = Crude neuronal count per sq.cm of section.

Assay of SDH activity.

The SDH activity was recorded as previously described by (Fakunle et al., 2011). The tissues of Lateral geniculate body specimens were weighed and homogenized using glass homogenizer containing weight to volume ratio (1:1) of tissues to cold 1.1% potassium chloride with pestle. All operations were carried out at 0 to 4°C. The homogenates were centrifuged at $13000 \times g$ for 5 min using National centrifuge model Z230M. The supernatant fractions were frozen for 60 min (-70°C) and the second supernatant was used to assay for SDH. The activities involved the measurement of the rate of decolourization of 2, 6-dichlorophenol-indophenol dye in the presence of succinate and cyanide. Decrease in absorbance at wavelength 600 nm was followed by spectrophotometer for 10 min (10 readings) at one-minute interval. One unit of enzyme is defined as the amount that will catalyze one mole of substrate in one minute.

Statistical analysis

The data were analyzed using the computerized statistical package 'SPSS Version 11'. Mean and standard error of mean (SEM) values for each experiment 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 LGB to determine if there is any statistically significant difference

Table 1. (Mean±SEM)g body weight distribution at the end of experiment.

WEEKS	NO OF RATS	GROUPS			
		C	T1	T2	T3
0	10	154.8 ± 3.53	169.3 ± 7.00	190.8 ± 7.98	170.5 ± 01.86
1	10	163.8 ± 2.18	167.8 ± 8.06	185.6 ± 14.37	168.4 ± 03.24
2	10	178.3 ± 5.19	159.3 ± 6.97	173.0 ± 20.24	171.2 ± 07.01
3	10	191.3 ± 2.06	150.5 ± 4.94	166.6 ± 6.46	178.8 ± 05.82
4	10	197.8 ± 2.81	146.7 ± 4.98	156.4 ± 10.19	184.4 ± 10.79
5	10	207.8 ± 5.51	140.6 ± 6.80*	150.7 ± 13.92*	195.1 ± 10.53
6	10	213.3 ± 5.59	138.7 ± 6.63*	144.8 ± 14.53*	209.6 ± 10.20

*(P < 0.05) Significance difference when compared with control using t-test

Table 2. 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	1.84± 0.31	2.81	6	0.001
T1	10	1.63± 0.13 ^{††}			
T2	10	1.32± 0.44 [†]			
T3	10	1.80± 0.90 ^{††}			

[†]P< 0.001, Values are statistically significant

^{††}P> 0.001, Values are statistically insignificant

in absolute neuronal count between the control and treatment groups.

RESULTS

Body weight

Treatment group T₁ and T₂ showed significantly decreased body weight (P < 0.05) of mean±sem (138.7 ± 6.63 and 144.8 ± 14.53g) respectively compared to the control group of mean±sem (213.3 ± 5.59g). Treatment group T₃ showed an insignificant (P > 0.05) decrease in body weight at the end of the first week of acetaminophen administration with Mean±sem (168.4 ± 3.24g compared to the initial weight of Mean±sem (170.5 ± 1.86g). However, continuous administration of acetaminophen during the 2nd, 3rd, 4th and 5th week showed a gradual increase in body weight to the end of the 6th week as Mean±sem (209.6 ± 10.20)g. However, this was insignificant (P > 0.05) as compared to the control group as seen in Table 1.

Brain weights

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. The mean brain weight loss was insignificant (P>0.01) for group T3 of Mean±sem(1.79 ± 0.21)g when compared to the control group as seen in Table 2.

Neuronal density and Neuronal diameter

Treatment groups T1 and T2 had significantly (P<0.05) reduced neuronal density for the purkinje cells Mean±SEM (288.75±21.44 and 327.88±06.11)/sq.cm when compared to the control group with Mean±SEM (541.15±12.26)/sq.cm which translates to a percentage neuronal loss of 47% and 39% respectively for treatment groups T1 and T2. Although 7% neuronal loss was recorded for treatment group T3 with Mean±SEM (501.11±16.02)/sq.cm of neuronal cells, however this was statistically insignificant (P>0.05) when compared to the control group. The neuronal transverse diameter revealed statistically significantly reduced values (P<0.05) for treatment groups T1 and T2 of Mean±SEM (0.52±0.22 and 0.86±0.22)µm respectively compared to Mean±SEM(1.28±0.41)µm obtained from the control section. The values obtained for neuronal transverse diameter in treatment group T3 was Mean±SEM (1.16±0.51)µm which is insignificant P>0.05 as compared to the control group as seen Table 3.

Table 3. Mean \pm SEM (Neuronal density and cells diameter) of Pyramidal cells and percentage neuronal loss.

GROUPS	Neuronal cells/sq.cm	Neuronal cells Diamter(μ m)	Neuronal loss(%)
C	541.15 \pm 12.26	1.28 \pm 0.41	-
T1	288.75 \pm 21.44	0.52 \pm 0.22	46.64
T2	327.88 \pm 06.11	0.86 \pm 0.39	39.41
T3	501.11 \pm 16.02	1.16 \pm 0.51	07.40

Table 4. Mean \pm SEM of the Activities of SDH and Protein concentration in the Lateral Geniculate Body.

GROUPS	SDH ACTIVITY(IU/L)
C	862.28 \pm 5.80
T1	405.19 \pm 4.21
T2	308.73 \pm 3.29
T3	700.76 \pm 6.18

Biochemical Quantification

Treatment groups T1 and T2 had significantly ($P < 0.001$) reduced activities in SDH revealed Mean \pm SEM (605.19 \pm 4.21 and 708.73 \pm 3.29)IU/L respectively compared to the control group of Mean \pm SEM (1286.28 \pm 5.80)IU/L. However statistically insignificant ($P > 0.001$) increased values were obtained in group T3 with Mean \pm SEM (1000.76 \pm 6.18) IU/L as seen Table 4.

DISCUSSION

The results of this study revealed that simultaneous intake of acetaminophen and ethanol clearly affects the body weight, neuronal cellular distribution and activity of the enzymes of carbohydrate metabolism within the lateral geniculate body. The statistically significant ($P < 0.05$) mean body weight decrease seen in the treatment groups T1 and T2 as shown in Table 1 can be correlated with the pattern of ethanol consumption as shown in Figure 1 posting a pictorial representation of an initial decrease in volume of ethanol consumption in the first three weeks followed by gradual increase in the volume of ethanol consumed from the end of the third week and throughout the experiment. However, this pattern of ethanol consumption when viewed in relation to the quantity of feed consumed Table 2 showed that ethanol depressed feeding significantly ($P < 0.01$) in the treatment groups T1 and T2. The depressed feeding noticed here can be as result of a gradual process of ethanol dependence which is the need for continued alcohol consumption to avoid a withdrawal syndrome that generally occurs from 6 to 48 hours after the last drink. (Linnoila et al., 1987 and Morrow et al., 1988). Hence

gradually there appears to be more urge to drink ethanol than for the feed consumption. In other words the weight loss observed here is in conformity with the earlier reports of effects of alcohol on digestion, absorption, storage, utilization and excretion of essential nutrients such as vitamins, minerals and proteins (Lieber, 2003). Alcohol impairs nutrient absorption by damaging cells lining the stomach and intestines, and disabling transport of some nutrients into the blood (Feinman et al., 1998). Alcohol also inhibits the breakdown of nutrients into usable substances, by decreasing the secretion of digestive enzymes from the pancreas (Korsten, 1989).

Moreover, (Thomson and Pratt, 1992) reported that excessive alcohol intake can impair the utilization of nutrients by altering their storage and excretion. 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 (Artnak et al., 1998 and Bridger et al., 1998) but that similar serious liver damage may also occur with 'therapeutic' use (Andreu et al., 1999). In the original studies of the mechanisms of toxicity, paracetamol was found to cause liver damage through conversion by hepatic cytochrome P450 enzymes to a minor but toxic intermediate metabolite and this was subsequently identified as *N*-acetyl-*p*-benzoquinoneimine (Corcoran et al., 1980). In accordance with this mechanism, the susceptibility to paracetamol-induced liver damage in animals was increased when these enzymes were stimulated by prior administration of inducing agents such as phenobarbitone and 3-methylcholanthrene, and decreased by inhibitors such as piperonyl butoxide and 4-methylpyrazole (Thummel et al., 1989). The results of this study point to the fact that

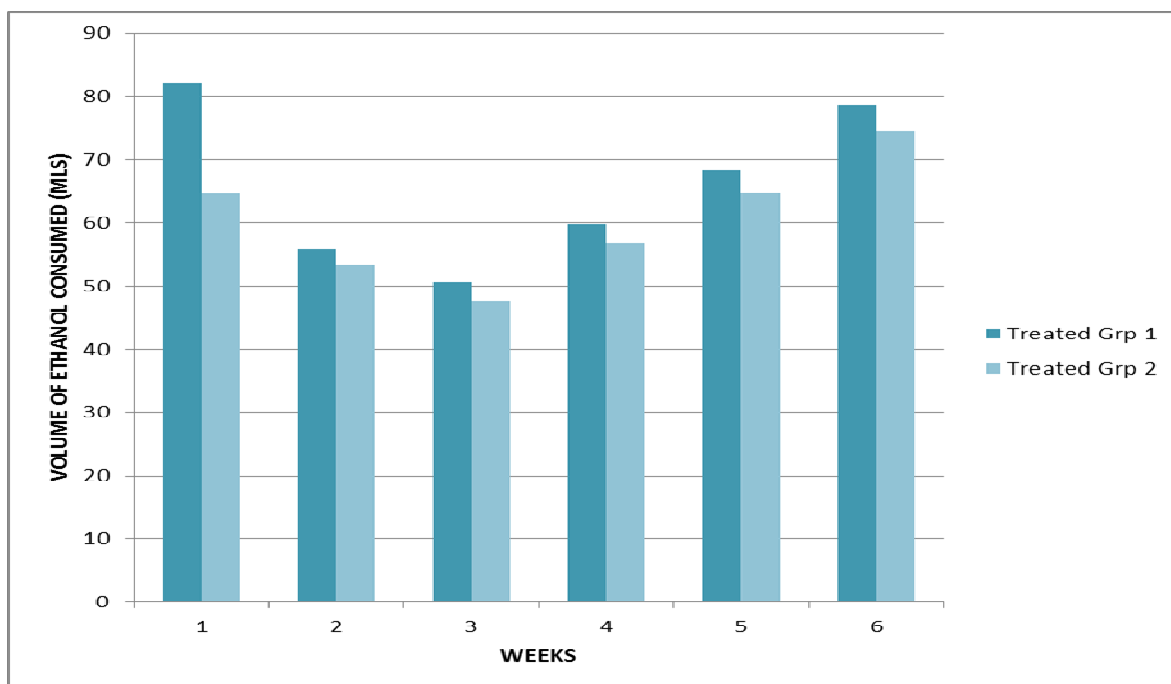


Figure 1. Histogram showing Ethanol consumption pattern at the end of administration.

chronic simultaneous administration of acetaminophen and ethanol had adverse effects on the brain weight and neuronal cell population of the lateral geniculate body. The shrunken brains reported in this study are in conformation with earlier findings of (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 when compared to the control group C as shown in Table 3 can be due to effects of acetaminophen and ethanol as findings have revealed that alcohol impairs nutrient absorption. Structural brain damage including shrinkage has also been credited to effects of chronic consumption of ethanol (Rosenbloom, 2001).

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 1 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 by (Ajibade et al., 2008) 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 (Henderson et al., 2000). 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 (Ledig et al., 1981). 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 (Cinthya and Rafael, 2004), 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 (Kerr et al., 1995). In this study, the mean diameters of the purkinje cells revealed significantly reduced values ($P < 0.05$) for treatment groups T1 and T2 compared to the control group and values obtained for neuronal transverse diameter for the

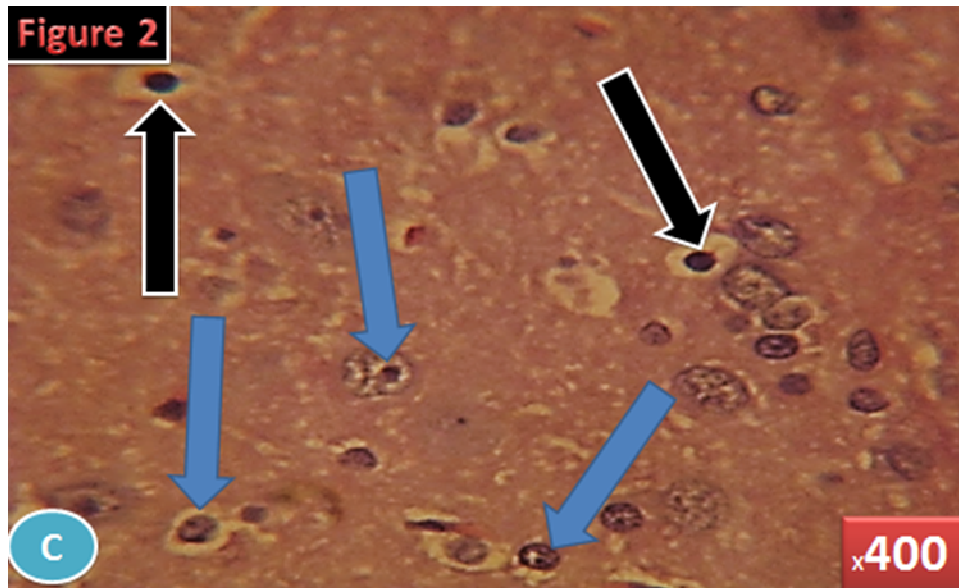


Figure 2. Photomicrograph of lateral geniculate body (LGB) (control section C) showing the distinctly distributed normal neuronal cells having located nuclei and nissl granules (blue arrows) and the glial cells (Black arrows) Cresyl violet stain x400.

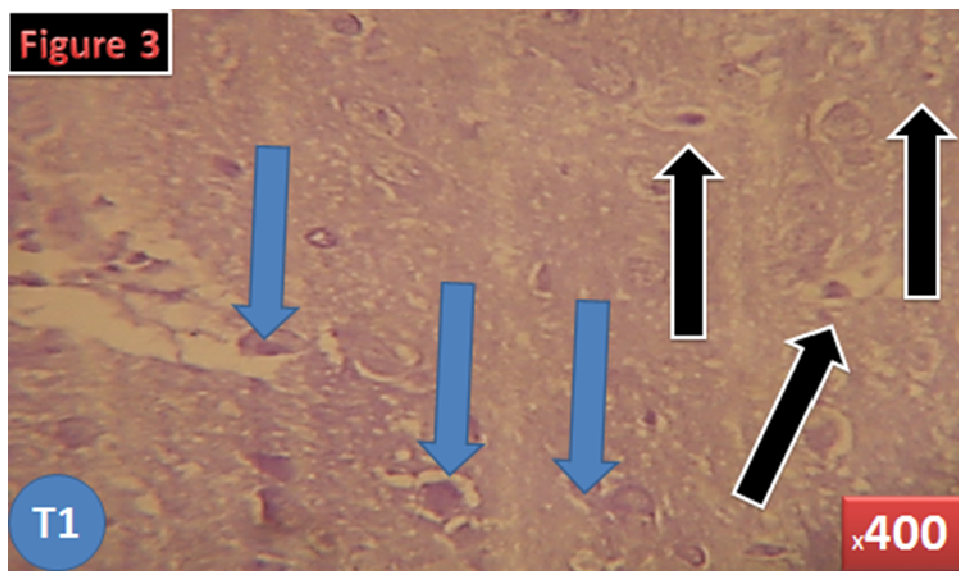


Figure 3. Photomicrograph of lateral geniculate body (LGB) (Treatment section T1) showing scantily distributed pyknotic neuronal cells with obliterated cell bodies (blue arrows) and the glial cells (Black arrows) Cresyl violet stain x400.

neuronal cells in treatment group T3 was 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 pictures seen in Figures 3 and 4 as compared to Figures 2 and 5 is apoptotic in nature confirming the earlier reports that ethanol triggers widespread apoptotic neurodegeneration in the brain (Ikonomidou et al., 2000) 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. Succinate dehydrogenase (SDH) is involved in energy production via the Krebs cycle. It is one of the major regulatory enzymes of Krebs cycle. The tricarboxylic acid (TCA) cycle, also known as the Krebs cycle, has long been considered so crucial for the basal metabolism of cell that no significant and primary defect

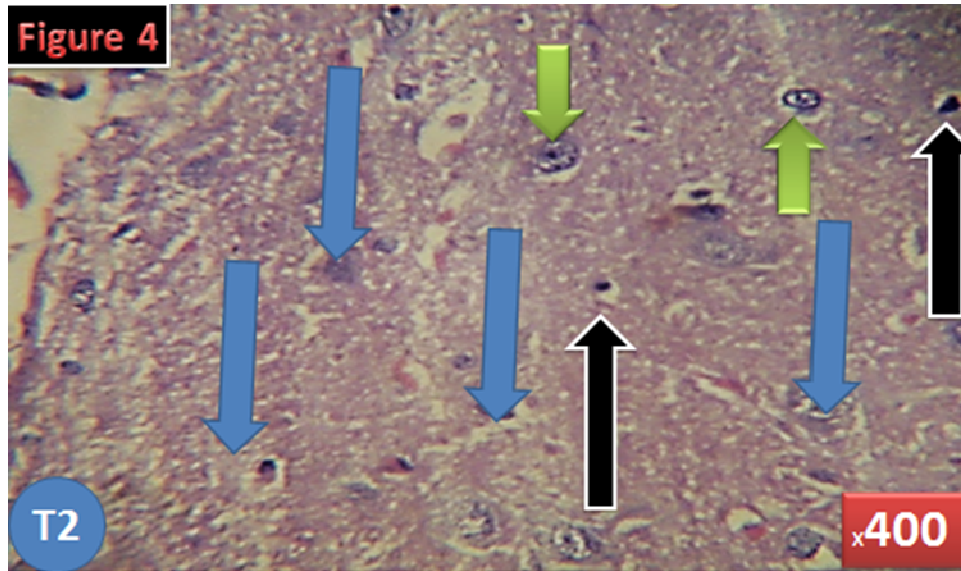


Figure 4. Photomicrograph of lateral geniculate body (LGB) (Treatment section T2) showing scantily distributed pyknotic neuronal cells with obliterated cell bodies (blue arrows) as well as few normal neurons (Green arrows) and the glial cells (Black arrows) Cresyl violet stain x400.

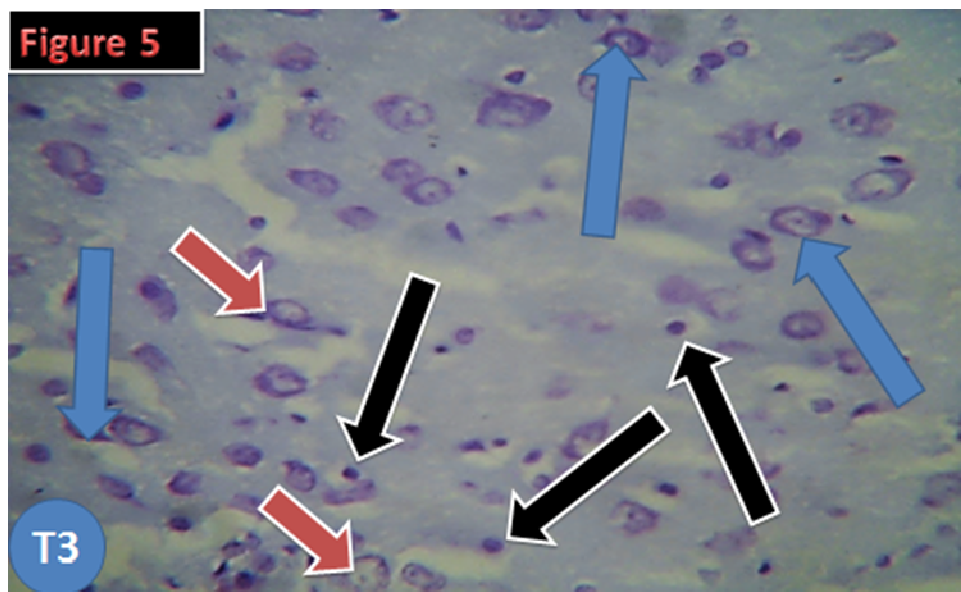


Figure 5. Photomicrograph of lateral geniculate body (LGB) (Treatment section T3) showing evenly distributed neuronal cells (blue arrows) with few obliterated neurons with clear cytoplasm (Green arrows) and the normal glial cells (Black arrows) Cresyl violet stain x400.

in any of its enzyme components could be compatible with life (Tyler, 1982 and Whelan et al, 1983). In the adult, the source of energy flow is through anaerobic glycolysis mediated through LDH and aerobic tricarboxylic citric pathway in which one of the important enzymes is succinic dehydrogenase (SDH). This enzyme participates in the citric acid cycle (Krebs cycle, or tricarboxylic acid cycle (TCA cycle)). It is the only enzyme of the cycle

found within the mitochondrial membrane. It catalyzes the reaction of succinate to fumarate with the simultaneous conversion of an FAD co-factor into FADH_2 . The membranous position of the enzyme facilitates FADH_2 access to the electron transport chain at the level of Coenzyme Q (Peters, 1982). The decreased SDH levels in treatment groups T1 and T2 as seen in Table 4 by the administered drugs shows its inhibition at the mitochond-

rial level which in the present study may be due to reduced oxygen uptake by the rats under chemical stress initiated by ethanol and potentiated by acetaminophen. The dysfunction of the mitochondrial respiratory chain, being direct intracellular source of reactive oxygen species (ROS) (Ajith et al., 2009) points to the inhibitory effects of ethanol and acetaminophen on SDH as observed in the lateral geniculate body, and thus presenting an adverse effect on carbohydrate metabolism, could result in energy shortage in the brain leading to impairment in neuronal activity. This observation may again point to adverse effects of ethanol and acetaminophen on the recoding function of lateral geniculate body as regards visual signals.

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