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PROTECTIVE EFFECTS OF L-THEANINE ON THE CO-ADMINISTRATION OF ETHANOL AND ALUMINUM IN THE LIVER OF MALE WISTAR RATS

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ABSTRACT

The study investigated possible protective role of L-theanine on toxicity caused by aluminum and ethanol. Thirty two (32) wistar rats were grouped into 4; with each group containing 8 rats. Group A which is the control group was administered distilled water. Group B was administered 0.4ml ethanol in 100g of rat and 0.25ml aluminum sulphate in 100g of rat; group C were administered 0.4ml ethanol in 100g of rat, 0.25ml aluminum sulphate in 100g of rat and 0.15ml of L-theanine in 100g of rat; group D was administered 0.4ml of ethanol in 100g of rat, 0.25ml of aluminum sulphate in 100g of rat and 0.2ml of L-theanine in 100g of rat and the administration lasted for 14 days. Growth rates were monitored and antioxidant content in the liver as well as liver histology was assayed. Results obtained show that theanine caused enhancement of growth while ethanol and aluminum caused growth depression from 8 days of the experiment compared to the control. Ethanol and aluminum caused significant reduction in liver (Superoxide dismutase (SOD), Glutathione (GSH), Glutathione Peroxidase (GPX), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Lipid Peroxidation (LPO) and Hydrogen Peroxide (H_2O_2); and also caused a significant alteration in antioxidants activities compared with the control. Treatment of rats with theanine caused appreciable ameliorative effect on the

induced liver damaged. It can be concluded that ethanol and aluminum caused significant damage to the liver through oxidative stress mechanisms; treatment with theanine has a mild protective effect on ethanol and aluminum induced toxicity.

Keyword: Antioxidant, Aluminum Sulphate, Ethanol, L-Theanine, Liver

1 Introduction

L-theanine is an antioxidant, also known as L-glutamylethylamide and N^5-ethyl-L-glutamine. In other word, it is an amino acid analogue of the proteinogenic amino acids L-glutamate and L-glutamine. It is found primarily in particular plant and fungal species. It was discovered as a constituent of green tea in 1949 and 1950, and was isolated from gyokuro leaves (Components of Gyokuro, 2015). In addition, L-theanine is a specific amide found in the tea plant (*Camellia sinensis*), the basidiomycete (*Boletus badius*) and Guayusatea (*Ilex guayusa*). It is synthesized from glutamine and ethylamine in the tea root and then transferred to the leaf where it accumulates (Borzelleca *et al*., 2006). It is one of the important components related to taste, and to the healthy function of tea (Morgan *et al*., 2010). Most studies have used L-theanine as a dietary supplement ingredient (Nobre *et al*., 2008). Furthermore, it provides a unique broth or savory flavor to green tea infusions, reduces the harmful impacts of alcohol in the liver where alcohol is converted to extreme toxic chemical referred to acetaldehyde, helps to boost the digestion of acetaldehyde and to block the harmful free radicals and function in the digestion of alcohol in the liver, preventing a lot of stress on the liver (Sadzuka *et al*., 2005). Ethanol, also called alcohol, ethyl alcohol, and drinking alcohol, is the principal type of alcohol found in alcoholic beverages. It is a volatile, flammable, colorless liquid with a slight characteristic odor (Green and Enama, 2015). Its chemical formula is C2H6O, which can be written also as $CH_3{-}CH_2{-}OH$ or $C_2H_5{-}OH$ (an ethyl group linked to a hydroxyl group), and is often abbreviated as EtOH (Rosso, 2012). It is one of the oldest and commonest recreational drugs, causing the characteristic

affect intoxication ("drunkenness") (Jones, 2008). Ethanol is toxic to the body when consumed in sufficient quantity. Upon consumption, it is immediately disposed out of the body. Over 90 percent of it is processed in the liver. In the liver, the alcohol dehydrogenase enzyme converts ethanol into acetaldehyde, which itself is toxic. Acetaldehyde is destroyed mostly immediately by the aldehyde dehydrogenase enzyme, which converts it to acetate ions. Aluminum is a trivalent cation found in its ionic form in most kinds of animal and plant tissues and in natural waters everywhere. The mass of Aluminum makes up 8% of the earth crust. The metal is so chemically reactive and limited. Due to its reactivity, aluminum in nature is found only in combination with other elements. Aluminum sulfate is a chemical compound with the formula $Al_2(SO_4)_3$. It is soluble in water and is mainly used as a coagulating agent (promoting particle collision by neutralizing charge) in the purification of drinking water and waste water treatment plants, and also in paper manufacturing (Robin *et al*., 2011). Aluminum sulphate is of two forms; both forms are soluble in water, non-combustible and non-toxic. Anhydrous aluminum sulphate is a white crystalline solid, and an aluminum salt with immune adjuvant activity. Thus, Aluminum is known to have neurotoxic effect that can result in oxidative damage to a range of cellular biomolecules, though the belief to be non-toxic and easily eliminated from the body, encourages its use in daily life.

The effect of toxicity on tissues is very great; toxic substances have many deleterious effects on the liver, kidney, lungs, heart and muscles; often times, they result in many clinical problems and generate free radicals which have the tendency of causing tissue damage. Aluminum toxicity occurs when a person breathes in high levels of aluminum from the air, or stores high levels of aluminum in the body. The symptoms of aluminum toxicity include muscle weakness, bone pain, fractures that do not heal, especially in ribs and pelvis, altered mental status, premature osteoporosis, anemia, impaired iron absorption, impaired maturity, dementia, seizures, delayed growth in children and spinal deformities such as scoliosis and

kyphosis. Theanine, an important amino acid, has an ameliorative (protective) effect on toxicity. It has also been reported to have psychoactive properties (Georgievics, 2013).

2 MATERIALS AND METHODS

Ethanol was obtained from the Biochemistry laboratory, Osun State University, Osogbo. L-Theanine was obtained from AK Scientific Inc., USA. Aluminum sulphate was purchased from Kadlad Nigeria Limited, Osogbo, Osun State. Top loading weighing balance (Ohaus Cooperation Pine Brook, England), UV Spectrometer (Unispec, USA), centrifuge (Surgifield centrifuge, England) and general glass wears. Diagnostic kits (ALT and AST are products of Randox Chemical Limited, England), antioxidant kits (SOD, GSH, GPX,) were obtained from Sayman Chemicals Michigan, United States of America. All other chemicals were analytical grade and purchased from British Drug House Poole, England and prepared in the laboratory using distilled water.

2.1 DESIGN EXPERIMENTAL

Thirty-two (32) albino wistar rats (average weight 120g) were obtained and put up at Osun State University's Central Animal House in Osogbo, Osun State. The rats were housed in ventilated wooden cages with a twelve hours light or dark cycle. The rats were fed standard rat pellets and water on an as- needed basis while the cages were washed twice daily. Before administering the drug, the rats were acclimatized for two weeks. The animals were cared for in relation with current laboratory animal ethical guidelines.

2.2 SAMPLE ADMINISTRATION

Thirty two (32) wistar The rats were divided into four groups of eight each. Distilled water was given to Group A, the control group. Group B received treatment with 0.4ml ethanol in

100g of rat and 0.25ml aluminum sulphate in 100g of rat; group C were administered with 0.4ml ethanol in 100g of rat, 0.25ml aluminum sulphate in 100g of rat and 0.15ml of L-theanine in 100g of rat; group D was administered with 0.4ml of ethanol in 100g of rat, 0.25ml of aluminum sulphate in 100g of rat and 0.2ml of L-theanine in 100g of rat. The administration which lasted for 14 days was done using the oral canula.

2.3 DETERMINATION OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY IN TISSUE

Misra and Fridovich's method was used to determine the level of SOD activity (1972).

A 1 in 5 dilution was made by diluting 0.2 ml of sample in 0.8 ml of distilled water. To equilibrate the spectrophotometer, an aliquot of 0.2 ml of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2), and the reaction began with the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture, which was quickly mixed by inversion. The reference cuvette was made up of 2.5 mL of buffer, 0.3 mL of substrate (adrenaline), and 0.2 mL of water. For 150 seconds, the absorbance at 480 nm increased every 30 seconds.

Per minute absorbance increase= $\frac{A_3 - A_0}{2.5}$

Where A_0 = absorbance after 30 seconds

A_3=absorbance after 150 seconds

$$\% \text{ inhibition} = 100 \times \frac{\text{Increase in absorbance for substrate}}{\text{Increase in absorbance for blank}}$$

The amount of SOD required to inhibit the oxidation of adrenaline by 50% was given as one unit of SOD activity.

2.4 GSH ACTIVITY DETERMINATION

In order to estimate the level of reduced glutathione, the method of Beutler et al. (1963) was used. An equal volume of 4 percent sulfosalicyclic acid was added to an aliquot of the homogenate to deproteinize it. This was centrifuged for 5 minutes at 4,000 xg. Following that, 0.5ml of supernatant was added to 4.5ml of Ellman reagent. With 0.5ml of the diluted precipitating agent and 4.5ml of Ellman reagent, a blank was prepared. At 412nm absorbance, the GSH level is corresponding.

2.5 ASSAY OF ASPARTATE AMINOTRANSFERASE (AST)

The AST was measured using a 1cm light path cuvette against a reagent blank. Hg549nm wavelength and 37oC incubation temperature were used. 0.5ml of RI was added to 0.1ml serum sample, and the mixture was incubated for 30 minutes at 37degreesC. 0.5ml of R2 was added and then incubated at 20-25degreeC for 20 minutes. After 5 minutes, 5ml of NaOH was added to the mixture, and the absorbance of the sample was measured against a blank sample.

2.6 ALANINE AMINOTRANSFERASE (ALT) ANALYSIS

The ALT was measured using a 1m light path cuvette against a reagent blank. Hg 546nm (530-550) wavelength, 370C incubation temperature 0.5ml of RI was mixed with 0.1ml of serum sample and incubated for 30 minutes at 370°C. 0.5ml of R2 was added and incubated at 20-25degreeC for 20 minutes. After 5 minutes, 5ml of NaOH was added to the mixture, and the absorbance of the sample was measured against a blank sample.

2.7 EVALUATION OF LIPID PEROXIDATION (LPO)

Varshney and Kale's method of measuring the level of Malondialdehyde produced during peroxidation was used to determine lipid peroxidation (1990). An aliquot of 400 microliters

of the sample was mixed with 1.6ml of tris-KCl buffer and 500 microliters of 30% TCA. After that, 500 microliters of 0.75 percent TBA were added and placed in a water bath at 800 degrees Celsius for 45 minutes. This was then cooled in ice and centrifuged for 5 minutes at 3000g. At 532nm, the absorbance of the clear supernatant was measured against a reference blank of distilled water. Lipid peroxidation was calculated using a molar extinction coefficient of 1.56 * 105 M-1 Cm-1 and expressed as MDA formed/mg protein or gram tissue.

$$\text{LPO (MDA formed/mg protein)} = \frac{\text{Absorbance} * \text{mixture volume}}{\text{mg protein} * E532nm * \text{volume of sample}}$$

(Mmol/mg protein)

2.8 LIVER HISTOPATHOLOGY

Tissues from the organs with a thickness of about 5mm were fixed in 10% neutralized buffered formalin. These tissues were histopathologically examined using the standard paraffin-wax embedding method. The tissues were dehydrated. Thereafter, clearing were carried out, this clearing involves replacing alcohol in the dehydrated tissue with a solvent of wax .The tissues were then, cut into sections and alcohols cleaned slides, smeared with albumin were used to float out the sections. After then, slides were kept overnight in an incubator, Haematoxylin and eosin staining; mounting and examination under a microscope.

3. RESULTS

3.1 Effects of the L-Theanine on the Co-administration of Ethanol and Aluminum Sulphate on Superoxide Dismutase (SOD) Concentration in the Liver

The influence of L-theanine on the co-administration of ethanol and aluminum sulphate on superoxide dismutase (SOD) concentration in the samples for groups A, B, C and D were determined. A substantial increase in superoxide dismutase concentration was observed in

groups C and D; but there was a slight decrease in group B when compared to the control. The end result is depicted in the diagram below.

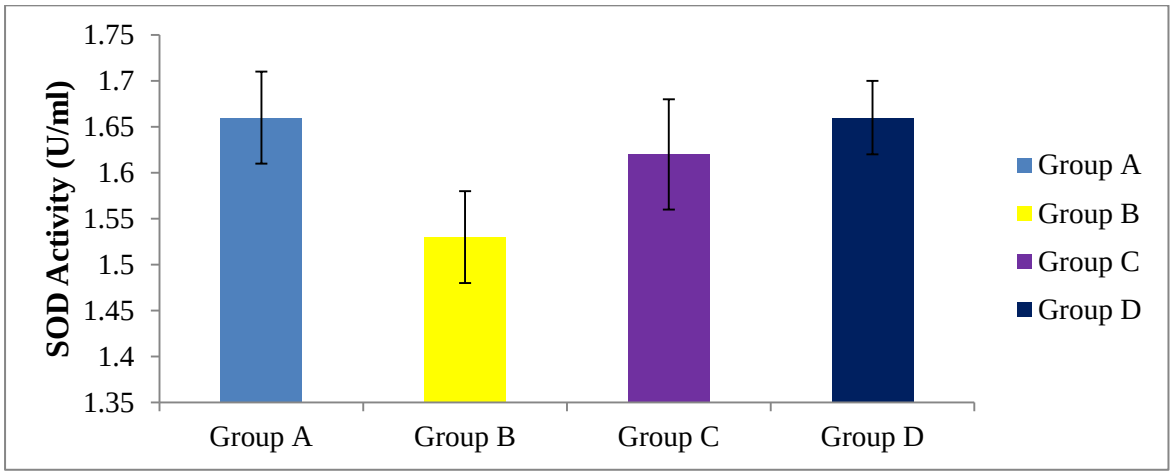

Figure.1: Diagram showing the influence of L- theanine on the co-administration of ethanol and aluminum sulphate on superoxide dismutase in samples A, B, C and D.

3.2 Influences of the L-Theanine on the Co-administration of Ethanol and Aluminum Sulphate on GSH Concentration in the Liver

Influences of the L-theanine on the co-administration of ethanol and aluminum sulphate on GSH concentration in the samples for groups A, B, C and D were determined. A significant increase in GSH concentration was observed in groups C and D; however, group B witnessed a mild decrease in comparison to the control. The end outcome is shown in the diagram below.

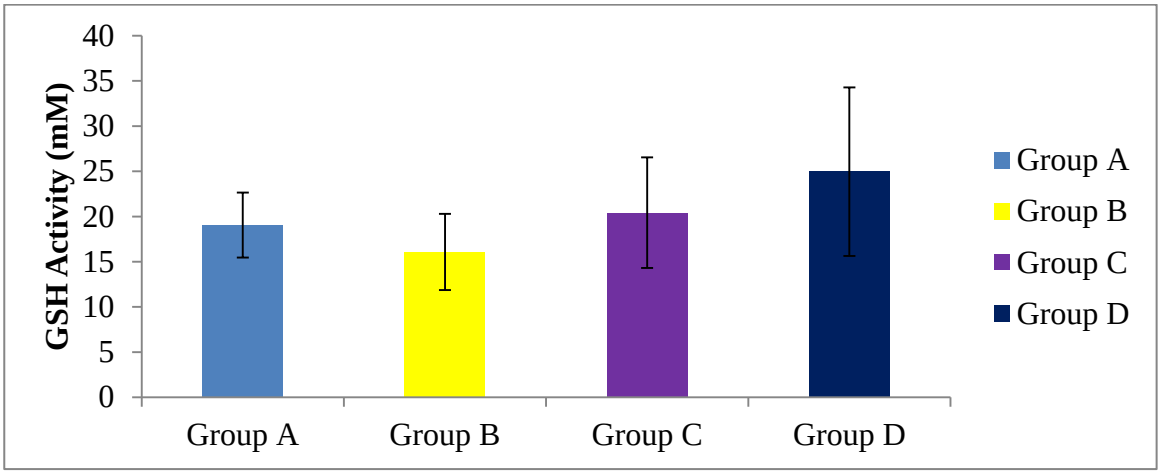

Figure 2: Diagram showing the L- theanine influence on the co-administration of ethanol and aluminum sulphate on GSH in samples A, B, C and D.

3.3 Effects of the L-Theanine on the Co-administration of Ethanol and Aluminum Sulphate on AST Concentration in the Liver

The L-theanine influence on the co-administration of ethanol and aluminum sulphate on AST concentration in the samples for groups A, B, C and D were determined. A mild decrease was observed in liver AST concentration in groups B; however, groups C and D witnessed a when compared to the control, there is a significant increase. The result is shown in the diagram below.

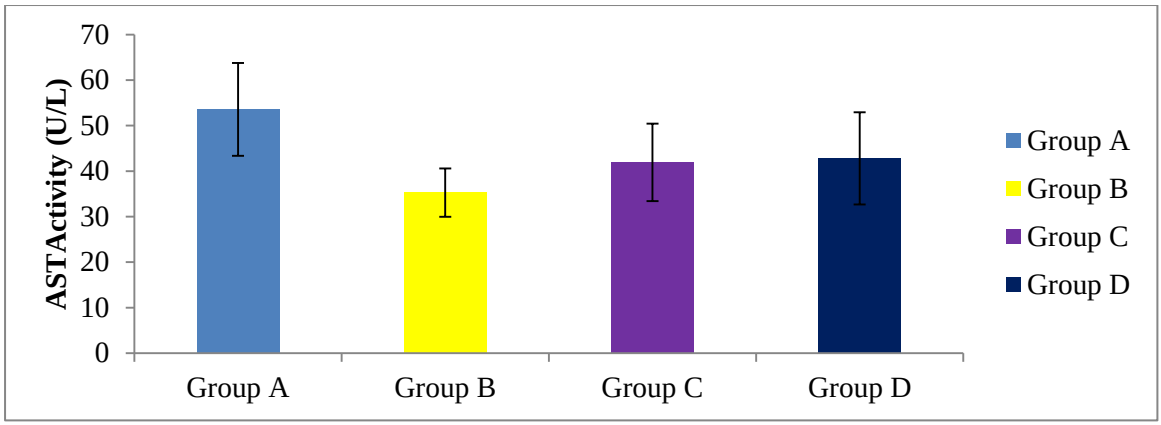

Figure 3: Diagram showing the influence of L- theanine on the co-administration of ethanol and aluminum sulphate on AST concentration in samples A, B, C and D.

3.4 Effects of the L-Theanine on the Co-administration of Ethanol and Aluminum Sulphate on ALT Concentration in the Liver

The L-theanine influences on the co-administration of ethanol and aluminum sulphate on ALT concentration in the samples for groups A, B, C and D were determined. Groups C and D witnessed a significant increase in liver ALT, however, when compared to the control, group B showed a slight increase.. The result is shown in diagram below.

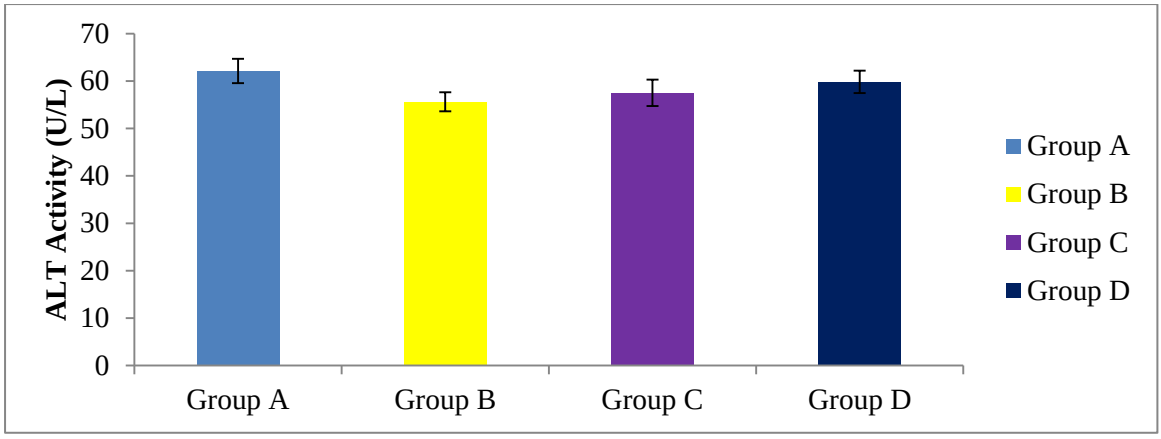

Figure 4: Diagram showing the influence of L- theanine on the co-administration of ethanol and aluminum sulphate on ALT concentration in samples A, B, C and D.

3.5 L- Theanine influences on the Co-administration of Ethanol and Aluminum in Lipid Peroxidation (LPO) Concentration in the Liver

The lipid peroxidation content of the liver samples for groups A, B and D were determined. Group B observed an increase in LPO liver, due to its pro-oxidant activity. Group C and D in comparism to the control, showed a lesser growth in LPO liver. The result is shown in the diagram below.

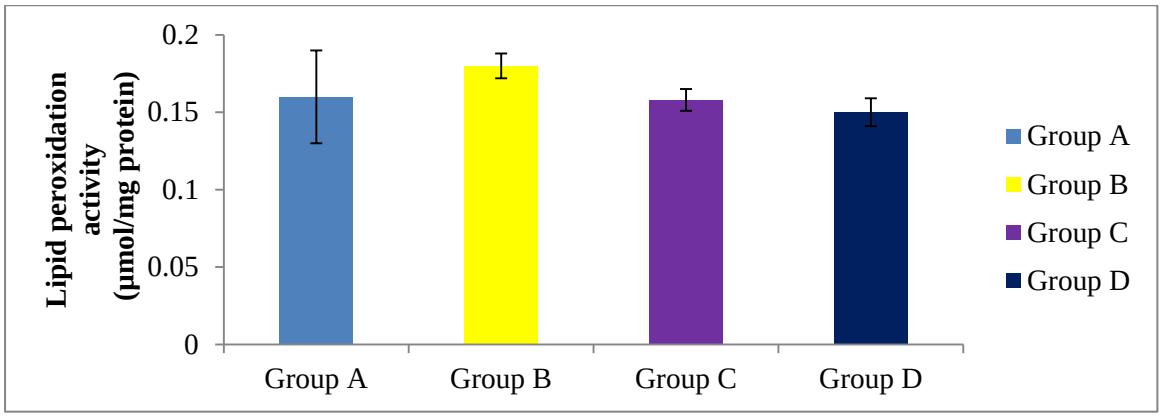

Figure 5: L-Theanine Influence on the co-administration of Aluminum Sulphate and ethanol on Lipid Peroxidation.

3.6 Influences of the L-Theanine on the Co-administration of Ethanol and Aluminum Sulphate and Liver Histology

Histopathological images of liver sections from Wistar rats given L-theanine, ethanol and aluminum sulphate is as shown in **Fig.6**.This section shows the effect of ethanol and aluminum sulphate, as well as protective effect of theanine during treatment. The sections in the diagram below, shows that there was no distortion of organelles, especially nucleus, in the plate A; while Plate B was observed to have more distortion of organelles (such as nucleus) due to the high effect of ethanol and aluminum sulphate on the liver tissue. Plate C shows a less distortion of organelles in the liver tissue due to theanine's protective effect on toxic liver tissue treated with L-theanine. Plate D shows lesser or no distortion of organelles (especially nucleus) due to the high protective effect of theanine on the toxic liver tissue treated with L-theanine.

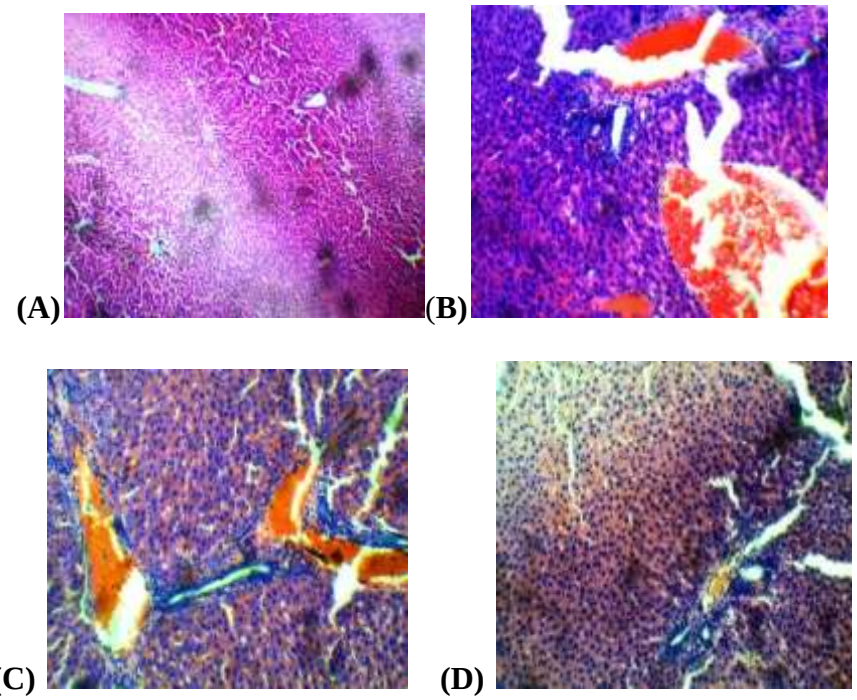

Fig.6 Histopathological images of wistar rat liver sections x400 magnification A: untreated control group B: pretreated with 5g/kg b.wt. ethanol and 20mg/kg b.wt. aluminum sulphate. C: pretreated with 5mg/kg b.wt. ethanol, 20mg/kg b.wt. aluminum, and 150mg/kg b.wt. L-theanine. D: pretreated with 5mg/kg b.wt. ethanol, 20mg/kg b.wt. aluminum, and 200mg/kg b.wt. L-theanine.

Plate A: shows the hepatic tissue with preserved normal lobular architecture. The portal tracts are unremarkable. There is a bile material within the blood vessel and sinusoids.

Plate B: shows hepatic tissue with mild distortion of the normal lobular architecture. The portal tracts are unremarkable where portal vein cystically dilated .There is marked interportal fibrosis with lymphocytic infiltration. There is a dilation of sinusoids in areas. Also seen are bile materials in some sinusoid and blood vessels.

Plate C: shows hepatic tissue with distorted normal architectural. The portal tracts are unremarkable with few dilated portal vein .There is a mild fibrosis of portal tracts. There is a piece meal necrosis of some peripheral hepatocytes. There is presence of bile material within the dilated blood vessels sinusoids.

Plate D: shows hepatic tissue with a less distorted normal architectural. The portal tracts are unremarkable and moderate necrosis of some peripheral hepatocytes. There is bile material within the blood vessel and sinusoids.

4. CONCLUSION

The effect of L-theanine on the treated rats caused an increase in the liver activity of the male wistar rats, when analyzed with biomarkers such as ALT and AST, along with SOD, GSH, except LPO. However, the untreated rats were observed to have liver disease due to their decrease in liver activity during analyses. Thus, it can be concluded that ethanol and aluminum caused significant damage to the liver through oxidative stress mechanisms; treatment with theanine has a mild protective effect on ethanol and aluminum induced toxicity.

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