Hepatoprotective Effect of Coconut Oil Against Aluminium Chloride and D-Galactose-Induced Hepatotoxicity in Male Wistar Rats

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Abstract-

Background: Damage or Injury to the liver as a result of exposure to certain harmful drugs known as hepatotoxins is referred to as hepatotoxicity, this condition is becoming highly alarming as it contributes to over two (2) million death annually and worldwide. After administering virgin coconut oil (VCO)to rat models of hepatotoxicity induced with Aluminium Chloride (AlCl3) and D-galactose (D-gal), serum levels of liver enzymes and histopathological alterations in the hepatic tissue were evaluated.

Objectives: The current study investigated the hepatoprotective effect of virgin coconut oil against AlCl3 and D-gal induced hepatotoxicity in male Wistar rats.

Materials and methods: Twenty eight (28) healthy male Wistar rats were used in this study. VCO (1 ml/kg and 3 ml/kg) was given orally to the AlCl3 and D-gal induced hepatotoxic rats for four (4) weeks. At the end of treatment, blood samples were collected for the determination of serum levels of liver enzymes. The liver was harvested processed and histopathological changes as result of the adminstration of AlCl3 and D-gal to the liver were also documented.

Results: VCO administration significantly raised the serum levels of globulin and total protein, while lowering alanine aminotransferase (AST) and aspartate aminotransferase (ALT) levels and also suppressing histopathological changes such as venular congestion, interlobular degeneration, presence of excess kupffer cells and fibrosis alterations in the liver.

Conclusion: Virgin coconut cil has a hepatoprotective effect against Aluminium Chloride and D-galactose-induced hepatotoxicity by attenuating histological changes and normalizing the serum levels of liver enzymes.

Indexed Terms- Virgin Coconut Oil, Hepatotoxicity, Histopathology, Aluminium Chloride and D-galactose.

I. INTRODUCTION

Liver disease contributes to about 2 million deaths annually and worldwide, with approximately 1 million deaths attributed to complications of liver cirrhosis and another million to viral hepatitis and hepatocellular carcinoma (Liao et al., 2016). Liver cirrhosis presently ranks the 11th most prevalent cause of death globally, while liver cancer stands as the 16th leading cause of death, which collectively constitute about 3.5% of all global deaths (Tajiri & Shimizu, 2013).

The liver is essential for sustaining the body's physiological equilibrium (homeostasis) (Ozougwu et al., 2017), its main function is to oversee and regulate the processing and safety of nutrients absorbed from the gut before they enter the bloodstream (Allen, 2014). Because the liver plays metabolic and detoxification functions in the body, and hence it is continuously and variedly exposed to xenobiotics, hepatotoxins, environmental pollutants, and chemotherapeutic agents (Andrade et al., 2019). Due to the liver's central role in the metabolism, detoxification and excretion of xenobiotic, it is made to be more susceptible to their adverse and toxic effects eventually leading to liver injury caused by various toxic chemicals hence leading to hepatotoxicity (Andrade et al., 2019 and Devarbhavi et al., 2023).

Hepatotoxicity, refers to damage or dysfunction of the liver cells (hepatocytes) which is associated with an overload of drugs, xenobiotics or generally hepatotoxins (the chemicals that cause liver injury). Drug-induced liver damage is a clinical consequence that can be challenging to recognize, avoid, and manage because liver disorders are mostly asymptomatic at the onset (Vishnumukkala et al., 2024).

During the process of liver detoxification, a group of enzymes known as drug metabolizing enzymes (DMEs) are activated in order to detoxify a drug or xenobiotic (non-self-exogenous substances unrecognized by the body). However, certain drugs may cause liver injury even when introduced within the therapeutic ranges. At times, hepatotoxicity may result not only from the direct toxicity of the hepatotoxicants but also from a reactive metabolite or from an immunologically-mediated response affecting the liver cells (Aftab et al., 2021), in cases and conditions where the natural protective mechanisms of the liver becomes overwhelmed during the process of detoxification and excretion of any hepatotoxins, the consequences are damage to the liver cells which will eventually result into hepatic injury and ultimately progress through several stages of liver disorder (Haider et al., 2015).

Aluminium (Al) is a heavy metal utilized in various aspects of human daily life, despite lacking any recognized biological significance in the human body, its widespread usage in developing countries has resulted in increased exposure to aluminum through various means. Al ranks the third most abundant element on earth, comprising approximately 8% of the total earth's crust. It is also the most commonly used and absorbed element in human daily activities, and exposure to Al occurs through multiple avenues as it is found abundant in deodorants, medications such as antacids, cosmetics, dietary sources, pesticides, and air pollution (Mahdi et al., 2021 and Vishnumukkala et al., 2024). Al is found to naturally occur in combination with other elements due to its high reactivity, forming compounds like Aluminum Chloride ($AlCl_3$) of which recent studies have established it toxicity on the liver, brain and other organs in the body (Mahdi et al., 2021 and Vishnumukkala et al., 2024).

D-galactose (D-gal) is a reducing sugar that simply reacts with the free amines of amino acids in peptides and proteins. Thus it been established as a senescence agent producing advanced glycation end products (AGEs) (Mahdi et al., 2021). Frequent administration of D-gal at low doses has proven to cause changes that mimic natural aging processes in animals, such as oxidative stress, decreased immune response, liver dysfunction and alterations in gene transcription (Allen, 2014). In addition, D-gal has also shown to cause acute hepatic necrosis and cirrhosis in rats during long-term administration (Li et al., 2018).

Findings have reported the pharmaceutical properties of virgin coconut oil (VCO) as compared to other oils. This includes its hepatoprotective, anti-inflammatory, antimicrobial, and anti-hyper-cholesterol effect. Moreover, it also helps to improve the antioxidant level and control the lipid peroxidation process (Nevin & Rajamohan, 2004). Therefore, VCO is gaining more attention due to its nutraceutical and pharmaceutical properties.

II. MATERIALS AND METHOD

CHEMICALS AND REAGENTS

AlCl3 and D-gal were freely obtained from Biochemistry laboratory of Gombe State University, normal saline which was used to dissolve both AlCl3 and D-gal was purchased from a Pharmaceutical Store in Gombe.

The Virgin Coconut Oil (VCO) was extracted using cold pressed extraction method according to Nevin and Rajamohan, 2004. Mature coconuts were purchased from commercial sellers in Gombe main market and were used for the extraction of VCO.

EXPERIMENTAL ANIMALS AND ETHICAL APPROVAL

Healthy male albino Wistar rats (10-12 months old) weighing about 150-180 grams were used in this study, the rats were obtained from the animal house of Human Anatomy Department of University of Jos, Nigeria. Rats were compartmentalized into four groups of seven in each, under the same laboratory condition of a 12-hour light and dark cycle, room temperature of 23 ± 1°C, and 50% humidity, and given unlimited access to distilled water and standard rats feed. The rats were acclimatized for 1-week before the commencement of the treatment which lasted for four weeks.

Before the commencement of the experiment, ethical approval was obtained from the ethical committee for the care and handling of laboratory animals of the College of Basic Medical Sciences Gombe State University, and rats were handled in humane manner according to the approved animal experimental procedures.

ANIMAL GROUPINGS AND EXPERIMENTAL DESIGN

1. Control group (which received only normal Rats feeds with physiological saline)
2. Model group (AlCl3 + D-gal of 200 mg/kg and 60 mg/kg respectively)
3. Low dose VCO group (model + VCO 1 ml/kg)
4. High dose VCO group (model + VCO 3 ml/kg)

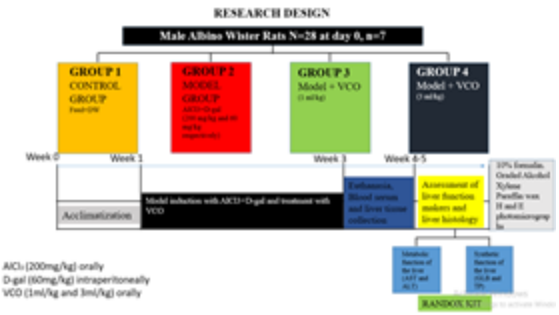

Figure 1. Animal grouping and experimental design.

BLOOD AND TISSUE SAMPLES COLLECTION

The treatment lasted for four (4) weeks, following the end of treatment, the rats were fasted for 24 hours and were then euthanized by cervical decapitation, and plain tube containers were used to collect blood sample for assessment of liver function makers.

Blood samples collected were centrifuged (3000g for 15 min) for separation of serum, the liver was also dissected out, washed using sodium chloride solution, and the liver sample collected from each rat was fixed in 10% formalin solution, for histopathological examination.

SERUM ASSESSMENT OF LIVER FUNCTIONS MAKERS

Liver function test to assess the liver function makers for metabolic and synthetic functions of the liver was carried out using RANDOX REAGENT KIT, and the liver function markers, alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB) and total protein (TP) were analyzed in serum sample stored at 4 ºC.

TISSUE PROCESSING AND HISTOPATHOLOGICAL EXAMINATION

For qualitative analysis of liver histology, the tissue samples collected were fixed in 10% buffered formalin for 24 hours, dehydrated in ethanol and embedded in paraffin wax, Sections of the liver tissue were prepared with the use of a rotary microtome and stained with Hematoxylin and Eosin (H and E) for microscopic study of histopathological alterations. The prepared slides were examined and photomicrographs were taken with MoticTM compound light microscope.

STATISTICAL ANALYSIS

Statistical analysis was performed using GraphPad Prism version 10.2.0, and all values were expressed as mean ± standard error of mean (SEM), the Comparison of evaluated parameters was carried out using One-way analysis of variance (ANOVA) followed by Turkey's post hoc test. And p-value < 0.05 was considered statistically significant.

III. RESULTS

SERUM ANALYSIS

IMPACT OF VCO ON SERUM LEVEL OF LIVER FUNCTION MARKERS (ALT, AST, GLB AND TP).

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) enzymes of metabolic product of the liver were significantly found to be in higher concentration in the serum of rats treated with AlCl3 and D-gal when compared to that found in serum level of rats in the control group which signifies Hepatotoxicity. However, when AlCl3 + D-gal-treated group were co-administered with VCO at doses of 1 ml/kg and 3 ml/kg, the serum levels of AST and ALT were significantly decreased when compared to AlCl3+ D-gal- exposed rats, as shown in (Figure 2A and 2B).

Additionally, the serum levels of liver synthetic markers Globulin (GLB) and Total Protein (TP) have also been observed to be significantly lower in serum of rats treated with AlCl3 + D-gal, However, while these rats have been co-administered with VCO at doses of 1 ml/kg and 3 ml/kg, the serum concentration of both GLB and TP significantly increased as compared to that in AlCl3 + D-gal induced rats as shown in (Figure 2C and 2D).

HISTOPATHOLOGICAL STUDY

IMPACT OF VCO ON THE LIVER HISTOLOGY

In normal control group, histological study showed a normal liver histology affirmed by presence of well-structured lobules, defined cords of hepatocytes, clear sinusoidal spaces, central vein located in the center of lobules and portal triad showing it content also (bile duct, hepatic artery and portal vein) as shown in (Figure 3A)

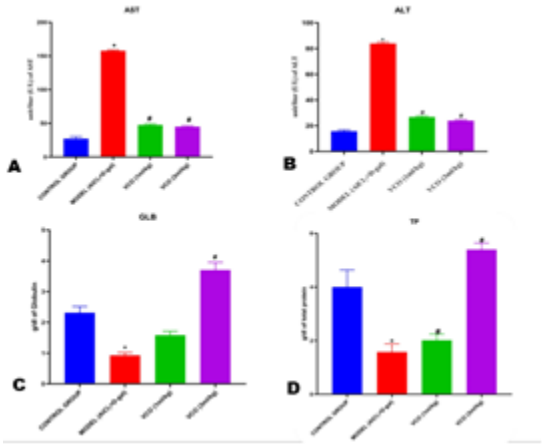

Figure. 2: Represents serum level of liver function markers of experimental rats. (A) AST; (B) ALT; (C) GLB; and (D) TP. Data were expressed as mean ± SEM, n = 7, *p < 0.05 versus control group; #p < 0.05 versus AlCl3 + D-gal.

In contrast, rats exposed to AlCl3 + D-gal showed a distorted liver histology with a pathological hallmarks of hepatotoxicity characterized by blood congestion in both portal and central veins, excess presence of kupffer cells within the sinusoidal area and thickening of the venular wall as shown in (Figure 3B)

Alternatively, low dose VCO treated group showed a moderate and also a considerable improvement in liver histology characterized by decrease in level of blood congestion within the portal and central vein and relatively clear sinusoidal spaces as shown in (Figure 3C). While the high dose VCO treated group denotes the hepatoprotective effect of VCO, by markedly improving the histoarchitecture of the liver histological features which in comparison looks almost exactly as that of the control group as shown in (Figure 3D).

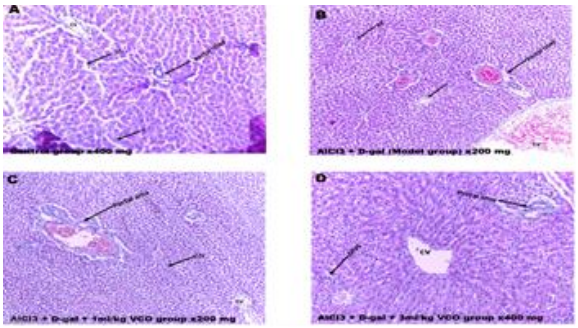

Figure 3. Photomicrographs of the hepatic tissues. (A) The model group shows the central vein (CV) surrounded by hepatocytes (H), sinusoids (S), and portal triad as well; (B) the AlCl3 + D-gal group shows a pathological hallmark of hepatotoxicity, the CV and portal vein (PV) with blood congestion, more dilated blood sinusoids, and the presence of excess Kupffer cells; (C) the AlCl3 + D-gal + 1ml/kg VCO group shows narrow blood sinusoid spaces, and the presence of few Kupffer cells with a bit clear portal triad; (D) the AlCl3 + D-gal + 3ml/kg VCO group exhibits fewer dilated blood sinusoids, and the presence of few Kupffer cells with a good histology which is almost as same as that of the control group. H&E

IV. DISCUSSION

Drug-induced hepatotoxicity is one of the major reasons for mortality and morbidity in human beings across the world (Braet et al., 2002). Recent studies have established the combined administration of AlCl3 + D-gal as one of the most common hepatotoxic compound used to induce liver injury in experimental model used for the screening of the hepatoprotective effect of various drugs (Singh et al., 2014). Our finding is consistent with this position. Further, another study has also explored the pharmacological importance of VCO such as anti-inflammatory, antioxidant and it hepatoprotective effects in rat models (Sreevallabhan et al., 2020).

Evidences of AlCl3 + D-gal induced hepatotoxicity have been linked to marked elevation in the serum level of liver enzymes (AST, ALT and ALP), increase in Bilirubin level and marked decrease in the serum level of Globulin (GLB), Albumin (ALB) and Total Protein (TP) (Anita et al., 2011). Results from this current study confirmed the toxic effect of AlCl3 + D-gal by prominent alterations in serum levels of liver damage markers (ALT, AST, GLB and TP) because acute liver injury alters hepatic transport function and membrane permeability, leading to leakage of marker enzymes (ALT and AST) from the cells, depresses the synthetic function of the liver, and pathologic alterations in liver histology (Famurewa et al., 2017)

In the present study, my results clearly demonstrated that AlCl3 + D-gal intoxication triggers significant increase in serum levels of AST and ALT while GLB and TP decreased. Thus, indicating compromised hepatic synthetic function and this corroborate previous reports from systematic investigations in rat models of liver toxicity (Vishnumukkala et al., 2024 and Min et al., 2015).

Histomorphological changes in the liver cells of rats induced with AlCl3 + D-gal has also shown blood congestion in both central and portal veins. These are the hallmarks of oxidative stress in liver, leading to portal hypertension, marginal necrosis and inflammation of hepatocytes, increase sinusoidal spaces and excess presence of kupffer cells along the sinusoidal and portal areas (Li et al., 2018).

The findings of this research work established the hepatotoxic effect of combined administration of AlCl3 + D-gal at 200 mg/kg and 60 mg/kg respectively as proven by a marked elevation in the serum level of liver enzymes (AST and ALT) in the model group (AlCl3 + D-gal only) compared to the normal control group. Also, the synthetic function of the liver was shown to be affected as proven by a marked decrease in the serum level of Globulin (0.93 g/dl) and Total Protein (1.58 g/dl) compared to the control group (2.32 g/dl and 4.00 g/dl respectively).

Further, the histological finding of this study also proved the hepatotoxicity of AlCl3 + D-gal as seen by extreme blood congestion in both portal and central vein of the model group (AlCl3 + D-gal only) followed by thickening of the venous wall which agrees with pathological findings according to (Li et al., 2018 and Vishnumukkala et al., 2024).

Again, our result has demonstrated the hepatoprotective effect of VCO against AlCl3 + D-

gal induced hepatotoxicity seen by the significant reduction in the serum level of liver enzymes (AST and ALT) virtually close to that of the normal control group as depicted in Figure 2 and 3 which is in accordance with the work of Abbasi et al., in 2021.

VCO was also shown to enhance the synthetic function of the liver as shown by a marked elevation in the serum level of Globulin and Total Protein, where the low dose VCO treated group showed a marked elevation in the serum level of Globulin (1.58 g/dl) and Total Protein (2.01 g/dl) above that of the model group (0.93 g/dl and 1.58 g/dl respectively). Similarly, the high dose VCO treated group showed an elevated serum level of Globulin (3.70 g/dl) and Total Protein (5.40 g/dl) even above that of the normal control group (2.32 g/dl and 4.00 g/dl respectively) as seen in figure 4 and 5. This result obtained coincided with findings of Famurewa et al., in 2017 and Narayanankutty et al., in 2018 where VCO was administered to rats.

The low dose VCO treated (1 ml/kg) groups showed moderate improvement in liver cytoarchitecture compared to the model group, which was also similar to histology of the control group with reduced blood congestion in the portal vein.

Whereas, high dose VCO treated group (3 ml/kg) showed virtually the same histology with that of the control group due to presence of clear central and portal veins.

CONCLUSION

In conclusion, the findings of this research has established the hepatoprotective effect of VCO in AlCl3 + D-gal induced hepatotoxicity by suppressing the serum level of liver enzymes (AST and ALT) and markedly increasing the serum level of Globulin and Total Protein. In addition, VCO also demonstrated hepatoprotective effectiveness by clearing congestion of blood clogs in both portal and central veins as revealed by histopathological studies.

However, the main cause resulting in histopathological symptoms of micro vesicular steatosis characterized by small droplets of fats between hepatocytes is not clearly known.

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REFERENCES

[1] Abdulmaguid, N. Y. M., & Hemdan, D. I. (2017). Hepatoprotective Activity of Avocado and Coconut Oils against Biochemical Alteration and Oxidative Stress Induced by Nicotine in Male Albino Rats. Information: an international Interdisciplinary journal, 20(10), 7463-7478.

[2] Aftab, A., Hina Mawani, G., Nabi P., Abdul-Samad, S., & Umair A.S. (2021). Hepatoprotective Role of Virgin Coconut Oil and Neem Extract in Acetaminophen Induced Liver Toxicity. Journal of medical science and public health DOI: https://doi.org/10.53350/pjmhs2115123209

[3] Andrade, R.J., Chalasani, N., and Björnsson, E.S. (2019). Drug-induced liver injury. Nat Rev Dis Primers 5, 58 https://doi.org/10.1038/s41572-019-0105-0

[4] Braet, F., and Wisse E., (2002). Structural and functional aspects of liver sinusoidal endothelial cell fenestrae: A review. Comp Hepatology, 1:1. https://doi.org/10.1186/1476-5926-1-1

[5] Deheragoda, M. (2022). Normal Liver Anatomy and Introduction to Liver Histology. Textbook of pediatrics Gastroenterology, Hepatology and Nutrition. Springer, Cham. https://doi.org/10.1007/978-3-030-80068-0_56.

[6] Devarbhavi, H., Asrani, S. K., Arab, J. P., Nartey, Y. A., Pose, E., & Kamath, P. S. (2023). Global burden of liver disease: update. Journal of hepatology, 79(2), 516–537. https://doi.org/10.1016/j.jhep.2023.03.017.A

[7] Đurašević, S., Jasnić, N., Prokić, M., Grigorov, I., Martinović, V., Đorđević, J., & Pavlović, S. (2019). The protective role of virgin coconut oil on the alloxan-induced oxidative stress in the liver, kidneys and heart of diabetic rats. Food &

function, 10 4, 2114-2124 https://doi.org/10.1039/c9fo00107g.

[8] Elsharkawy, A.M., Oakley, F., & Mann, D.A. (2014). The role and regulation of hepatic stellate cell apoptosis in reversal of liver fibrosis. Apoptosis. 10:927–939.

[9] Fabrellas, N., Carol, M., Torrabadella, F., & de Prada, G. (2018). Nursing care of patients with chronic liver diseases. Journal of Advanced Nursing, 74 (3), 498-500. doi: 10.1111/jan.13350.

[10] Ferrell L. (2002). Liver pathology: cirrhosis, hepatitis, and primary liver tumors. Update and diagnostic problems. Mod Pathology.; 13:679–704.

[11] Gasmi, B., & Kleiner, D. E. (2020). Liver Histology: Diagnostic and Prognostic Features. Clinics in liver disease, 24(1), 61–74. https://doi.org/10.1016/j.cld.2019.09.004.

[12] Grønkjær, L.L., & Lauridsen, M.M. (2021). Impact of liver disease on patient's quality of life: a mixed methods systematic review protocol. Journal of clinical medicine: JBI Evidence Synthesis 19(6): p 1412-1417. DOI: 10.11124/JBIES-20-00185.

[13] Haider, S., Liaquat, L., Shahzad, S., Sadir, S., Madiha, S., Batool, Z., Tabassum, S., Saleem, S., Naqvi, F., & Perveen, T. (2015). A high dose of short term exogenous D-galactose administration in young male rats produces symptoms simulating the natural aging process. Journal of life sciences, 124, 110-9. https://doi.org/10.1016/j.lfs.2015.01.016.

[14] Hartmut, J., Gregory, J., Gores, A.I., Cederbaum, J.A., Hinson, D.P., & John J.L. (2022) Mechanisms of Hepatotoxicity, Toxicological Sciences, Volume 65, Pages 166–176, https://doi.org/10.1093/toxsci/65.2.166.

[15] Hassani, M. (2022). Liver Structure, Function and its Interrelationships with Other Organs: A Review4. 88-92. 10.35629/5252-04018892. International journal of medicine and pharmacy: https://doi.org/10.15117/jbcpp-2013-0986.

[16] Kamri, A.M., Rahmawati, L.W., & Rahman, S. (2021). Systematic review: The effect of virgin coconut oil as a source of antioxidant. International journal of research and analytical review (IJRAR): www.ijrar.org (E-ISSN 2348-1269, P-ISSN 2349-5138)

[17] Krishnan, M. (2018). Anatomy and Histology of the Liver of Etroplus maculatus (Bloch). International Journal of Scientific and Research Publications (IJSRP). https://doi.org/10.29322/IJSRP.8.12.2018.P8436.

[18] Leila, A., Shirin, E., Amir, Z., Mohammad, J.M., Amin, Z., & Ali A.B. (2019). Removal of heavy metals by conventional water treatment plants using poly aluminum chloride, Toxin Reviews, 38:2, 127-134, DOI: 10.1080/15569543.2018.1431676.

[19] Li, C., Cheng, Z., Zhang, K., Wu, Z., & Zhang, L. (2018). Hepatotoxicity of D-galactose and its potential relevance to the pathogenesis of liver damage. Journal of Clinical and Translational Hepatology, 6(1), 50-55.

[20] Liao, C., Chen, B., Chiang, H., Chen, C., Chen, M., Ke, C., Wang, Y., Lin, W., Wang, C., & Lin, Y. (2016). Optimizing a Male Reproductive Aging Mouse Model by d-Galactose Injection. International Journal of Molecular Sciences, 17. https://doi.org/10.3390/ijms17010098.

[21] Liu, L., Wang, X., Hu, R., & Zhu, G. (2020). D-galactose-induced brain aging model: A systematic review and meta-analysis on cognitive outcomes and oxidative stress indices. Aging Clinical and Experimental Research, 32(1), 39-52.

[22] Lu, H., Lei, X., Zhang, Q., & Ma, Z. (2018). D-galactose induces necroptotic cell death in neuroblastoma cell lines. Journal of Cellular and Molecular Medicine, 22(6), 3074-3084.

[23] Mahdi, O., Chiroma, S.M., Baharuldin, M.T.H., MohdNor, N.H, Mat Taib, C.N., Jagadeesan, S, Devi, S., & Mohd, M.M.A. (2021). WIN55,212-2 Attenuates Cognitive Impairments in AlCl3 + D-Galactose-Induced Alzheimer's Disease Rats by Enhancing Neurogenesis and Reversing Oxidative Stress. Biomedicines. https:// doi.org/10.3390/biomedicines9091270.

[24] Min, J. Y., Min, K. B., Kim, D. S., & Paik, Y. K. (2015). Aluminum chloride-induced liver injury in rats: the protective effect of taurine. Environmental Toxicology, 30(10), 1151-1159.

[25] Narayanankutty, A., Palliyil, D., Kuruvilla, K., & Raghavamenon, A. (2018). Virgin coconut oil reverses hepatic steatosis by restoring redox homeostasis and lipid metabolism in male Wistar rats. Journal of the science of food and agriculture, 98 5, 1757-1764. https://doi.org/10.1002/jsfa.8650.

[26] Nevin, K.G. & Rajamohan, T. (2004). Beneficial effects of virgin coconut oil on lipid parameters and in vitro LDL oxidation, Clinical Biochemistry. 37 830–835.

[27] Nouri, S.G., & Sharif, M.R., (2014). Hemostatic effect of aluminum chloride in liver bleeding: an animal model study. Tehran University Medical Journal TUMS Publications, 72, 435-442.

[28] Otuechere, C., Madarikan, G., Simisola, T., Bankole, O. & Osho, A. (2014). Virgin coconut oil protects against liver damage in albino rats challenged with the anti-folate combination, trimethoprim-sulfamethoxazole. Journal of Basic and Clinical Physiology and Pharmacology, 25(2), 249-253. https://doi.org/10.1515/jbcpp-2013-0059.

[29] Ozougwu, J.C., & Eyo, J.E. (2014) Hepatoprotective effects of Allium cepa extracts on paracetamol-induced liver damage in rat. African Journal of Biotechnology 13(26): 2679 -2688.

[30] Sadek, K. M., Saleh, E. A., Nasr, S. M., & Nasr, H. M. (2018). The potential protective effect of ascorbic acid against aluminum chloride-induced hepatotoxicity and nephrotoxicity in rats. Environmental Science and Pollution Research, 25(2), 1617-1628.

[31] Scaglione S, Kliethermes S, Cao G, Shoham D, Durazo R, Luke A, (2015). The epidemiology of cirrhosis in the United States: a population-based study. J Clin Gastroenterol 2015; 49:690–696.

[32] Schuppan, D., & Afdhal, N. H. (2008). Liver cirrhosis. Lancet (London, England), 371(9615), 838–851. https://doi.org/10.1016/S0140-6736(08)60383-9

[33] Silalahi, J. (2020). Nutritional values and health protective properties of coconut oil. Indonesian Journal of Pharmaceutical and Clinical Research, 3(2), 1-12.

[34] Simorangkir, T. L., & Marpaung, O. S. (2023). Virgin Coconut Oil (VCO) Attenuates Hepatotoxicity Induced by Cigarette Smoke in Rats. Majalah Obat Tradisional. https://doi.org/10.22146/mot.82659.

[35] Tajiri, K., & Shimizu, Y. (2013). Liver physiology and liver diseases in the elderly. World journal of gastroenterology, 19(46), 8459–8467. https://doi.org/10.3748/wjg.v19.i46.8459.

[36] Tsochatzis, E. A., Bosch, J., & Burroughs, A. K. (2014). Liver cirrhosis. Lancet (London, England), 383(9930), 1749–1761. https://doi.org/10.1016/S0140-6736(14)60121-5.

[37] Vishnumukkala, T., Gopalakrishna, P. K., Karikalan, B., Jagadeesan, S., Baharuldin, M. T., Hidayat, B., and Thomas, W. (2024). Protective effect of Centella asiatica on AlCl3 and D-galactose induced hepatotoxicity in rats through the alleviation of oxidative stress as demonstrated by histological changes in liver. Royal College of Surgeons in Ireland. Journal contribution. https://hdl.handle.net/10779/rcsi.25021142.v1.

[38] Wanless, I.R., Nakashima, E., Sherman, M. (2000) Regression of human cirrhosis. Morphologic features and the genesis of incomplete septal cirrhosis. Arch Pathology Lab Med.; 124:1599–1607.

[39] Wu, X. N., Xue, F., Zhang, N. (2024). Global burden of liver cirrhosis and other chronic liver diseases caused by specific etiologies from 1990 to 2019. BMC Public Health 24, 363 https://doi.org/10.1186/s12889-024-17948-6.

[40] Xu, F., Liu, Y., Zhao, H., Yu, K., Song, M., Zhu, Y., & Li, Y. (2017). Aluminum chloride caused liver dysfunction and mitochondrial energy metabolism disorder in rat.. Journal of inorganic biochemistry, 174, 55-62. https://doi.org/10.1016/j.jinorgbio.2017.04.016.

[41] Yang, L.L. (2021). Anatomy and Physiology of the Liver. In: Milan, Z., Goonasekera, C. (eds) Anesthesia for Hepatico-Pancreatic-Biliary Surgery and Transplantation. Journal of hepatology Springer, Cham. https://doi.org/10.1007/978-3-030-51331-3_2

[42] Younossi, Z., and Henry, L. (2015). Overall health-related quality of life in patients with end-stage liver disease. Clin Liver Dis 2015; 6:9–14. Hepatology 2019;17:2085–92.

[43] Younossi, Z., Koenig., A. B., Abdelatif, D., Fazel, Y., Henry, L., & Wymer, M. (2016). Global epidemiology of nonalcoholic fatty liver disease. Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016; 64:73–84.

[44] Yousef, M. I., El-Demerdash, F. M., & Kamel, K. I. (2007). Alleviation of aluminum-induced oxidative stress in the liver and kidney of rats by the black seed (Nigella sativa L.). Journal of Biochemical and Molecular Toxicology, 21(4), 237-244.