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Effect of Ethanol Leaf Extract of *Annona senegalensis* on Lipid Profile and Kidney Function in Diethylnitrosamine-Induced Hepatocellular Carcinoma in Rats

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Abstract

Liver cancer remains a major global health burden, with hepatocellular carcinoma (HCC) being the most common form and a leading cause of cancer-related deaths. Its progression is often accompanied by metabolic and renal dysfunction, complicating treatment outcomes. While conventional therapies such as chemotherapy, radiotherapy, and targeted drug treatments offer benefits, they are frequently associated with high toxicity and adverse side effects. This discussion highlights the potential of *Annona senegalensis* as a safer alternative for managing HCC-related complications, particularly in the context of lipid metabolism and renal function. Evidence from experimental studies suggests that the ethanolic leaf extract of *A. senegalensis* significantly improves lipid profiles by reducing cholesterol levels from 368.57 ± 1.72 in the negative control group to 266.40 ± 0.88, 217.46 ± 0.82, and 182.24 ± 1.20 at 200, 400, and 600 mg/kg doses, respectively, while maintaining

favorable HDL levels at higher doses. These findings indicate the extract's potential to enhance lipid metabolism, lower cardiovascular risk, and provide renal protection during liver cancer progression. Beyond its biochemical effects, the study reinforces the growing relevance of integrating traditional medicinal plants into modern healthcare frameworks as complementary therapeutic options. Overall, *A. senegalensis* demonstrates promising hepatoprotective, metabolic, and nephroprotective effects, warranting further investigation into its bioactive compounds and clinical applicability.

Keywords: Ethanol; Leaves; *Annona senegalensis*; Lipid Profile; Kidney Function; Diethylnitrosamine; Hepatocellular Carcinoma; Wistar Rats

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most prevalent forms of liver cancer worldwide, accounting for a significant proportion of cancer-related deaths (Sayiner *et al.*, 2019). It is often associated with underlying liver diseases, such as hepatitis infections, cirrhosis, and exposure to carcinogenic compounds like diethylnitrosamine. It a malignant tumor that arises from hepatocytes, the major cell type in liver. (Luch, A. 2005), HCC is the most common primary hepatic tumor and the fifth most common tumor worldwide. It has ahigh incidence in sub-Saharan Africa and Asia. The HCC 5-years survival rate is less than 5 percent without treatment. Any chronic inflammatory liver disease has the potential to induce HCC, but the pathophysiological process found in up to 80 percent of cases of the disease is cirrhosis. (Sies*, et al.*, 2017), Approximately 90 to 95 percent of these tumors are the biologic consequences of persistent hepatitis B-virus (HBV) and hepatitis C virus (HCV) infections. (Basso*, et al.,* 2008), Certain diseases other than chronic Hepatitis B or C are associated with increased HCC incidence; iron overload cirrhosis (hemochromatosis), long standing alcoholic cirrhosis, alpha1-antitrypsin deficiency, and tyrosinemia. The disease is often clinically silent until it is well advanced or tumor diameter exceeds 10cm. Given the poor prognosis and lack of effective therapies for hepatocellular carcinoma, prevention programs are desperately needed. Surgical resection is the treatment of choice for patients with HCC when the tumor is small and limited to one lobe of the liver. (Halliwell, *et al.*, 2015) In cases where the tumor is larger or involves more than one lobe of the liver such that it cannot be removed, liver transplantation has also been per-formed. In either case, the cure rate averages only 20-30 percent, which has somewhat limited the use

of liver transplantation for this problem. Surveillance for HCC inpatients with cirrhosis may lead to an improved survival in cohort studies. Diethylnitrosamine (DEN) is a type of nitrosamine, a known hepatocarcinogen forming DNA adducts in the liver (Tolba *et al.*, 2015). The induction of oxidative stress by DEN further contributes to the pathogenesis of hepatocellular carcinoma. DEN and other known hepatocarcinogens induce hepatic injury by enhancing the proliferation of cells, which is accompanied by hepatocellular necrosis (You *et al.*, 2021). They target the liver where they are bio-transformed via the cytochrome p450-dependent mechanism, CYP2E1, explicitly resulting in DNA- adducts (Tolba *et al.*, 2015). Hepatic carcinogenesis is ranked the fifth most prevalent cancer and is usually influenced by agents such as alcohol, phenobarbital, 2-acetylaminofuorene, and DEN (Unsal and Belge-Kurutas, 2017).

Animal models are viewed as a critical tool for studying hepatic carcinogenesis and are often used for cancer research. Studies have reported early induction of oxidative stress, inflammation, and proliferation by DEN in rat models (Mansour *et al.*, 2019). When injected into young mice, DEN targets the liver, where it is biologically transformed into alkylating agents that can create mutagenic DNA adducts by centrilobular hepatocytes (Connor *et al.*, 2018). Additionally, there is proof that inflammation plays a role in the development of hepatocarcinogenesis brought on by DEN (Xu *et al.*, 2021). In addition to being a genotoxic substance, DEN is also hepatotoxic and causes necrotic cell death. This damage sets off an inflammatory response that increases the expression of mitogens such as interleukin-6 (IL-6), which encourages the proliferation of remaining hepatocytes as a form of reparative action (Hou *et al.*, 2018).

Medicinal plants and herbs have been a cornerstone of traditional medicine for centuries and have gained significant attention in modern pharmacology due to their bioactive compounds (Izah *et al.*, 2024). They contain phytochemicals and natural sources of antioxidants. *Annona senegalese* (AS) is of one such plant containing an abundance of phytochemicals; it contains alkaloids, flavonoids, glycosides, tannins, saponins, steroids, and anthocyanins (Musa *et al.*, 2017). These bioactive compounds have been reported to exhibit anticancer activities by inducing apoptosis, inhibiting angiogenesis, and modulating oxidative stress pathways. AS is often referred to as wild soursop; the plant is a 2 5 m tall shrub having blue to greenish, oblong, alternate, and simple leaves. It is prominent among the Northern region of Nigeria and commonly called in Hausa language, Gwandan da`ajìì (Okhale *et al.*, 2016).

Almost all the plant parts have been reported useful for medicinal applications. The leaves are used for yellow fever, tuberculosis, and smallpox treatment while the stem bark is employed for treating hernia and snakebite (Hanrahan, 2022). The root is employed to ease swallowing, impotence, and an antidote for poison; root bark helps in the treatment of infectious diseases while the juice from the plant dries up chickenpox on the skin (Emmanuel and Mamoudou, 2015). Several studies have documented its biological activities such anticonvulsant, antimicrobial, antiplasmodial, anti venomous, spermatogenic, anti-inflammatory, analgesic and antihelmintic activities (Jada *et al.* 2015).

Statement of Problem

Conventional cancer treatments, including chemotherapy, radiation therapy, and surgery, have been the cornerstone of cancer management for decades. However, they present significant challenges. Cancer treatments are often expensive, making them inaccessible to many patients, especially in low- and middle-income countries. The cost of chemotherapy, targeted therapy, and immunotherapy can run into thousands of dollars per treatment cycle. Traditional therapies often lead to side effectssuch as nausea, vomiting, fatigue, immunosuppression, and organ toxicity. For example, chemotherapy drugs like cisplatin can cause nephrotoxicity (kidney damage), while doxorubicin is associated with cardiotoxicity. Some cancers develop resistance to chemotherapy over time, reducing treatment effectiveness. Additionally, certain types of cancer, like liver cancer (hepatocellular carcinoma), respond poorly to available treatments, leading to high recurrence rates and poor prognosis. Liver diseases, particularly liver cancer and cirrhosis, often result in lipid metabolism disturbances and kidney dysfunction. These complications arise due to. The liver plays a central role in lipid metabolism, including cholesterol synthesis, fatty acid oxidation, and lipoprotein production. Liver cancer and related diseases can lead to dyslipidemia, characterized by increased triglycerides, altered cholesterol levels, and fatty liver accumulation. Hepatorenal syndrome (HRS) is a severe kidney condition associated with liver failure. It results from altered blood flow, inflammation, and oxidative stress, leading to progressive renal impairment. Despite the widespread use of herbal treatments in traditional medicine, their effects on lipid and kidney function in liver diseases remain underexplored. Herbal medicines often contain bioactive compounds with antioxidant, anti-inflammatory, and hepatoprotective properties, which could offer therapeutic benefits for managing these complications. However, scientific validation is limited, and further studies are needed to establish their efficacy and safety. Annona

senegalensis, commonly known as African custard apple or wild soursop, is a medicinal plant widely used in African traditional medicine. It has been reported to possess various pharmacological activities, including. preliminary studies suggest that extracts of Annona senegalensis may have cytotoxic effects on cancer cells, potentially inducing apoptosis (programmed cell death) and inhibiting tumor growth. Some studies on related species in the Annona genus have shown potential lipid-regulating effects, but there is limited direct evidence on *Annona senegalensis* and its role in modulating lipid profiles in liver cancer. While some traditional uses suggest nephroprotective effects, scientific studies evaluating its impact on kidney function in liver cancer models are scarce. given the growing interest in herbal medicine for cancer management, more research is needed to determine the mechanisms of action, optimal dosages, and potential toxicity of *Annona senegalensis* ethanol leaf extract in liver cancer models.

Justification of the Study

Liver cancer is a significant global health challenge, with metabolic and renal dysfunction often accompanying its progression. Conventional treatments such as chemotherapy, radiotherapy, and targeted drug therapies can be effective, but they are often associated with severe side effects and toxicity. As a result, researchers are increasingly exploring natural plant-based treatments as potential alternatives due to their bioactive compounds, which exhibit antioxidant, anti-inflammatory, and hepatoprotective properties. One such plant, Annona senegalensis, has gained scientific interest for its potential in cancer management and metabolic regulation. This discussion explores how Annona senegalensis may offer a safer alternative for managing liver cancer-related metabolic and renal dysfunction. Liver cancer, particularly hepatocellular carcinoma (HCC), significantly alters metabolic processes and renal function due to the liver's central role in metabolism and detoxification. As the disease progresses, patients often develop conditions such as:

Metabolic Dysregulation. Liver cancer disrupts carbohydrate, lipid, and protein metabolism, leading to conditions such as insulin resistance, cachexia (muscle wasting), and hyperlipidemia. Oxidative Stress and Inflammation: The cancerous liver undergoes excessive oxidative stress due to an imbalance between reactive oxygen species (ROS) production and antioxidant defenses. Renal Dysfunction: Liver cancer can contribute to hepatorenal syndrome, a severe condition in which kidney function deteriorates due to

altered blood circulation and inflammatory mediators. Given these complications, plant-based treatments with antioxidant, anti-inflammatory, and hepatoprotective properties may help mitigate these effects and support overall health. *Annona senegalensis*, also known as the African custard apple or wild soursop, is a medicinal plant used in traditional African medicine to treat various ailments. The plant contains a wide range of bioactive compounds, including: Alkaloids, Flavonoids Tannins, Saponins and Phenolic compounds. These phytochemicals contribute to its antioxidant, anti-inflammatory, antimicrobial, and anticancer effects. Oxidative stress plays a crucial role in liver cancer progression and associated metabolic dysfunction. The flavonoids and phenolic compounds in *Annona senegalensis* have free radical scavenging activity, reducing oxidative damage to liver cells. This can help. Protecting hepatocytes from ROS-induced damage. Enhancing the body's natural antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase). Reducing lipid peroxidation, which contributes to hepatic and renal dysfunction. Chronic inflammation is a key driver of liver cancer progression. Studies indicate that Annona senegalensis extracts contain compounds that inhibit pro-inflammatory cytokines such as TNF-α, IL-6, and NF-ϰB, thereby reducing inflammation in the liver and kidneys. These effects may: Protect liver tissues from fibrosis and cirrhosis, common precursors to cancer. Improve hepatic metabolic function by restoring enzyme activity. Prevent kidney damage associated with cancer-induced inflammation. Several studies suggest that Annona senegalensis has cytotoxic effects on cancer cells. Acetogenins, alkaloids, and flavonoids in the plant have been shown to induce apoptosis (programmed cell death) and inhibit proliferation of cancerous cells. These mechanisms make it a potential complementary therapy in liver cancer treatment. Annona senegalensis may also play a role in metabolic regulation by. Enhancing glucose metabolism, potentially reducing insulin resistance. Lowering lipid levels, which can help in managing hepatic steatosis (fatty liver), a risk factor for cancer. Modulating kidney function, reducing proteinuria (excess protein in urine) and improving glomerular filtration. The study of *Annona senegalensis* in liver cancer treatment contributes to a broader understanding of how plant-based therapies can complement or even replace conventional treatments. Scientific research on this plant could help. Validate traditional medicine practices by providing evidence-based support for its medicinal use, to develop new pharmacological formulations based on its bioactive compounds, reduce reliance on toxic chemotherapy drugs, which often lead to severe side effects such as nephrotoxicity and hepatotoxicity. By understanding the mechanisms through which

Annona senegalensis exerts its effects, researchers could isolate specific bioactive compounds and develop novel anti-cancer drugs. This could open avenues for targeted therapies that minimize harm to healthy tissues while effectively combating cancer cells. Since Annona senegalensis is widely available in Africa and other tropical regions, promoting its use as a complementary or alternative medicine could provide affordable and accessible treatment options, particularly in low-resource settings where access to conventional therapies is limited.

Aim

The primary aim of this research was to evaluate the effects of ethanol leaf extract of *Annona senegalensis* on lipid profile and kidney function in DEN-induced hepatocellular carcinoma in rats.

Objectives of the Study

i. To assess the impact of *Annona senegalensis* on lipid profile markers (total cholesterol, LDL, HDL, triglycerides) in DEN-induced HCC.

ii. To determine the effects of *Annona senegalensis* on kidney function markers (creatinine, urea, sodium, potassium chloride and co_2).

iii. To compare the effects of *Annona senegalensis* with standard treatment (Silymarin 100 mg/kg).

Hepatocellular Carcinoma (HCC)

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and is a leading cause of cancer-related deaths worldwide. It typically arises in the setting of chronic liver disease, particularly in patients with cirrhosis due to chronic hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol-related liver disease, or non-alcoholic fatty liver disease (NAFLD). Hepatocellular carcinoma (HCC) is a primary tumor of the liver and constitutes more than 90% of the primary tumor of the liver. Hepatocellular carcinoma occurs in approximately 85% of patients diagnosed with cirrhosis.(Ioannou *et al.,* 2007) HCC is now the fifth most common cause of cancer worldwide.(Ferlay *et al.*,2012) The second leading cause of cancer death after lung cancer in men is HCC.(Ferlay *et al.*, 2012) Five-year survival of HCC is 18% and second to pancreatic cancer.(Jemal *et al.*, 2017) Significant risk factors for hepatocellular carcinoma include viral hepatitis (hepatitis B and hepatitis C), alcoholic liver disease, and non-alcoholic liver steatohepatitis/non-alcoholic fatty liver

disease. HCC occurs in 80%-90% of patients with cirrhosis. The annual incidence of HCC in patients with cirrhosis is 2-4%. (Ioannou *et al.,*2007)

Diethyl nitrosamine (DEN)

Diethyl nitrosamine is a potent carcinogenic compound extensively used in scientific research to induce liver cancer (hepatocellular carcinoma, HCC) in experimental animal models. This compound mimics liver cancer development in humans, making it a valuable tool for studying cancer pathogenesis, progression, and potential therapeutic interventions. The role of DEN in hepatocarcinogenesis can be understood by examining its causes, risk factors, and global prevalence.

Causes: How DEN Induces Liver Cancer

DEN is a nitrosamine compound that primarily exerts its carcinogenic effects through the following mechanisms:

Metabolic Activation and DNA Damage**:** Once administered, DEN is metabolized in the liver by cytochrome P450 enzymes (especially CYP2E1). This metabolic activation leads to the formation of highly reactive ethyl radicals, which interact with cellular macromolecules, particularly DNA. The interaction results in the formation of DNA adducts, such as O6-ethylguanine, which leads to mutations and genomic instability (Thoolen *et al*., 2012).

Oxidative Stress and Inflammation**:** DEN exposure causes an increase in reactive oxygen species (ROS), leading to oxidative stress. This oxidative stress damages hepatocytes, triggering chronic inflammation, which is a key factor in liver cancer development. Inflammatory cytokines, such as TNF-α and IL-6, promote cell proliferation, survival, and angiogenesis, further driving tumorigenesis (Shalaby *et al*., 2020).

Alteration of Signaling Pathways**:** DEN disrupts several molecular pathways, including the Wnt/β-catenin, NF-ϰB, and PI3K/Akt signaling pathways. These disruptions contribute to uncontrolled cell division, apoptosis resistance, and enhanced metastatic potential (Bai et al., 2021; He et al., 2015).

Epigenetic Modifications: DEN exposure can lead to epigenetic changes, such as DNA methylation and histone modifications, which silence tumor suppressor genes. Such changes promote liver cancer progression by increasing the expression of oncogenes (Pogribny & Rusyn, 2013).

Risk Factors Associated with DEN-Induced Liver Cancer

The likelihood of liver cancer development due to DEN exposure depends on several risk factors, including:

Dosage and Duration of Exposure**:** Higher doses and prolonged exposure to DEN significantly increase the risk of liver tumor formation. Chronic low-dose exposure may still lead to liver damage over time (Wei *et al.,* 2020).

Genetic Susceptibility**:** Certain animal strains exhibit greater susceptibility to DEN-induced hepatocarcinogenesis due to genetic predispositions. Variations in cytochrome P450 enzyme activity can influence the extent of DEN metabolism and carcinogenicity (Sanchez-Perez *et al*., 2005).

Age and Gender**:** Younger animals are generally more susceptible to DEN-induced liver cancer, as their liver cells are more actively dividing. Male animals tend to have a higher incidence of DEN-induced HCC compared to females due to differences in hormonal regulation (e.g., testosterone enhances susceptibility) (Feng *et al*., 2011).

Co-exposure to Other Carcinogens or Liver-damaging Agents**:** Simultaneous exposure to other hepatotoxic agents such as aflatoxins, alcohol, or high-fat diets increases the risk of liver cancer development. Synergistic effects between DEN and other toxins can accelerate cancer progression (Maroofi *et al*., 2017).

Diet and Lifestyle Factors**:** High-fat and high-calorie diets may enhance DEN-induced hepatocarcinogenesis by promoting lipid accumulation and metabolic dysfunction in liver cells. Chronic alcohol consumption further exacerbates liver injury and increases susceptibility to DEN-induced mutations (Zhang *et al.,* 2019).

Global Prevalence and Research Implications

Use in Research Models**:** DEN is widely used across the globe in liver cancer research, primarily in rodent models (rats and mice). These models help in understanding human hepatocarcinogenesis and testing potential anticancer therapies (Bai *et al*., 2021).

Relevance to Human Liver Cancer**:** While DEN exposure is not a primary cause of liver cancer in humans, the molecular mechanisms of DEN-induced hepatocarcinogenesis closely resemble those observed in human HCC. This makes DEN a valuable tool for studying liver cancer pathophysiology, tumor progression, and therapeutic responses (He *et al*., 2015).

Environmental and Occupational Exposure**:** Although DEN is primarily used in experimental settings, nitrosamines (including DEN) are found in tobacco smoke, processed meats, and contaminated drinking water. Chronic exposure to nitrosamines in certain populations may contribute to increased liver cancer risk (Farber *et al.*, 2019).

Global Liver Cancer Burden**:** Liver cancer, particularly HCC, is the third leading cause of cancer-related deaths worldwide. Major risk factors for human HCC include chronic hepatitis B and C infections, alcohol abuse, aflatoxin exposure, and metabolic diseases (e.g., non-alcoholic fatty liver disease). Understanding DEN-induced hepatocarcinogenesis can provide insights into preventing and treating liver cancer in humans (Bai *et al.*, 2021).

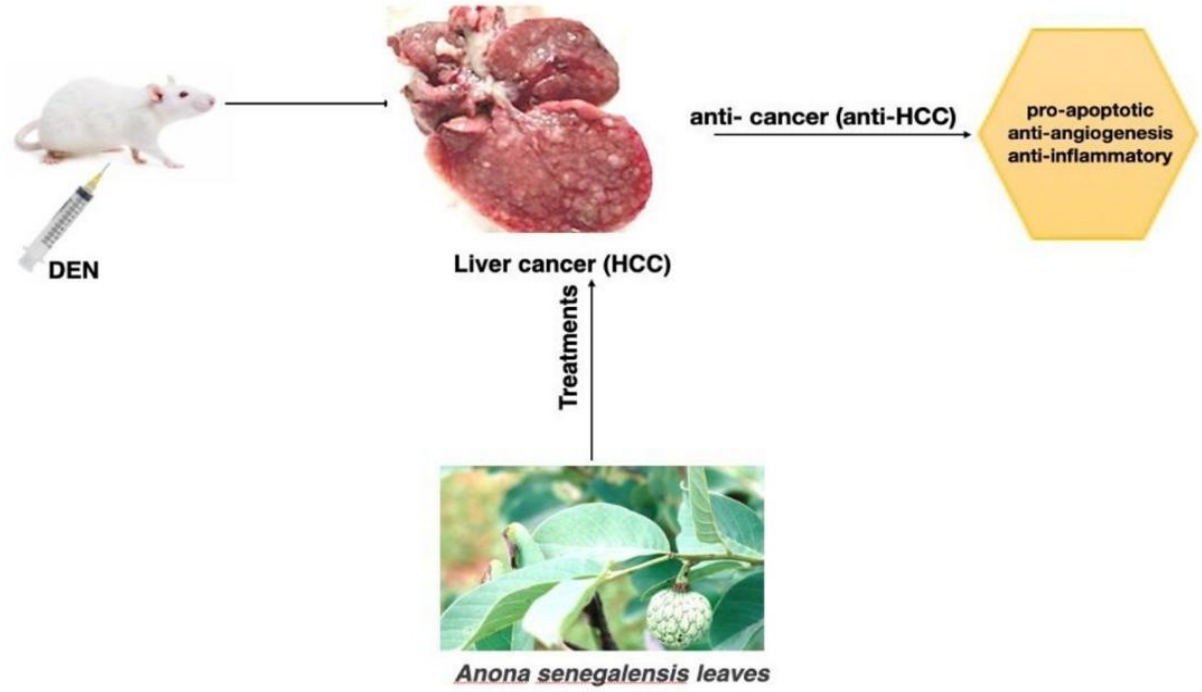

Fig 1. (Hepatocellular carcinoma) induce diethyl nitrosamine in Wistar rats.

Lipid Profile and Kidney Function in HCC

Two of the most vital organs in the body, the liver and kidneys filter toxins and control a number of metabolic functions to keep the body in a state of equilibrium, (Harris *et al.,* 2004). The kidneys are essential for filtering waste products and preserving fluid and electrolyte balance, while the liver is essential for detoxifying dangerous chemicals including narcotics, alcohol, and environmental pollutants. However,

exposure to environmental pollutants such as heavy metals can seriously harm these organs.

Liver cells play a serious task in the regulation of lipid metabolism. The principal location for lipoprotein and cholesterol synthesis is in the liver. In healthy to changes in the structure of lipoproteins and transport throughout the blood (Studenik *et al.,* 2000). Nevertheless, in cirrhotic patients, the metabolism of lipid is changed such that glycogen stores are significantly diminished, contributing to malnutrition and biolysis (Bassendine *et al.,* 2013). The preceding studies have reported that cirrhotic patients have abnormal metabolism of lipids, especially reduction in cholesterol level and hypobetalipoproteinemia (Napolitano *et al*., 2007). These alterations develop together with the progression of liver disease and may be considered as a prognostic marker for liver decompensation (Ghadir *et al.,* 2010). The maneuvers concerned with the decline of lipid particles in patients with cirrhosis are compound and needs multiple researches for a complete understanding. Protein microsomal triglyceride (TG) transfer protein − MTP], enzymatic (acyl CoA: cholesterol acyltransferase), and apoprotein (Apo AI) reduction are considered to be linked to that change (Tsai MH *et al.,* 2009). Owing to the wide occurrence of liver disease in Egypt, we made this study to assess the profile of lipids in cirrhotic patients and to evaluate the relationship between lipid profile and the degree of cirrhosis. Liver is the principal site for formation and clearance of lipoproteins. It receives fatty acids and cholesterol from peripheral tissues and diet, packages them into lipoprotein complexes and releases these complexes back into the circulation. (Pugh RN *et al.,* 1973) Hence it is not surprising that liver diseases can affect plasma lipid levels in a variety of ways. Chronic liver diseases due to various causes are often associated with dramatic reductions in plasma triglyceride and cholesterol level due to reduced lipoprotein biosynthetic capacity. (Andrikoula M *et al.,* 2006) Cholestasis is associated with hypercholesterolemia as the major excretory pathway of cholesterol is blocked in this disorder. Apart from the various complications seen in cirrhotic patients, chronic dyslipoproteinemia is one which can lead to alterations in cellular membrane lipids, that result in formation of abnormal RBCs, such as echinocytes, and alterations in membrane function with potential pathophysiologic consequences.

The liver is responsible for lipid synthesis, transportation, and degradation. It regulates: Cholesterol metabolism (synthesis and clearance)

Triglyceride levels (production and export via lipoproteins)

Lipoprotein processing (formation of HDL, LDL, and VLDL)

Fatty acid oxidation and synthesis (via beta-oxidation and lipogenesis)

Liver dysfunction, such as non-alcoholic fatty liver disease (NAFLD), cirrhosis, and hepatitis, leads to altered lipid metabolism, including:

Hyperlipidemia: Due to reduced clearance of lipoproteins, especially LDL and VLDL, leading to increased circulating triglycerides and cholesterol.

Dyslipidemia: Commonly presents as high LDL, low HDL, and elevated triglycerides, increasing cardiovascular risks.

Lipid accumulation in liver cells: Leads to hepatic steatosis, a hallmark of NAFLD and alcoholic liver disease.

Impact on Kidney Health

Liver dysfunction is strongly associated with kidney disease. Several mechanisms explain this relationship:

Inflammatory Response: Liver diseases often trigger systemic inflammation, contributing to kidney damage and leading to chronic kidney disease (CKD).

Oxidative Stress: Imbalance in oxidative stress and antioxidant defense systems due to liver dysfunction worsens renal health.

Endothelial Dysfunction: Liver disease affects vascular health, impairing kidney perfusion and glomerular function.

Impaired Lipid Clearance: Excess lipids in circulation contribute to lipotoxicity, promoting glomerular injury and tubulointerstitial damage.

Liver disease modifies several biomarkers used to assess lipid metabolism and kidney function:

Lipid Biomarkers

Increased Total Cholesterol and LDL-C: Due to impaired liver clearance.

Elevated Triglycerides: Hepatic dysfunction leads to decreased lipoprotein lipase activity.

Low HDL-C: Reduced liver function leads to impaired HDL formation and cholesterol efflux.

Increased VLDL: Defective metabolism results in elevated very low-density lipoproteins.

Renal Biomarkers

Elevated Serum Creatinine: Indicates impaired renal function, common in advanced liver disease.

Increased Blood Urea Nitrogen (BUN): Liver dysfunction alters nitrogen metabolism, affecting kidney clearance.

Proteinuria and Albuminuria: Often seen in patients with cirrhosis or NAFLD-related renal disease.

Decreased Glomerular Filtration Rate (GFR): Affected by systemic inflammation and lipid abnormalities.

Annona senegalensis

Annona senegalese, commonly referred to as African custard apple, wild custard apple, wild soursop, abo ibobo (in Yoruba), sunkungo (in Mandinka), and dorgot (in Wolof), belongs to the Annonaceae family. The species name senegalese signifies its origin from Senegal, where the type specimen was first collected (Romeiras et al., 2018). This plant serves as a traditional food source in Africa, with its fruits offering potential benefits such as enhanced nutrition, improved food security, rural development, and sustainable land management.

A. senegalese grows as either a shrub or a small tree, typically reaching heights between 2 and 6 meters, though it can occasionally grow up to 11 meters. Its bark varies from smooth to rough in texture and has a gray-silver or gray-brown hue, often marked with leaf scars and peeling in rounded flakes that reveal lighter underbark. Young branches are covered in thick, gray, brown, or yellowish hairs, which diminish with age (AgroForestry Tree Database, 2013). The leaves are green to blue-green, alternate, simple, and range from oblong to ovate or elliptic in shape, measuring between 6–18.5 cm in length and 2.5–11.5 cm in width. The upper surface of the leaves is mostly hairless, while the underside may be hairy, often displaying reddish aracnose veins. The leaf apex is either

rounded or slightly notched, with a squared or barely lobeliar base and an entire margin. The petioles are stout, measuring between 0.5–2.5 cm in length.

The flowers of A. senegalese grow singly or in clusters of two to four from the leaf axils, reaching up to 3 cm in diameter on 2 cm stalks (Okhale et al., 2016). Each flower has six thick petals arranged in two whorls, green on the exterior but either creamy or reddish inside, measuring around 0.8–1.5 cm in length and 0.9–1.1 cm in width. They may be smooth or slightly fuzzy. The inner whorl of petals curves over the stamens and ovary, while the three smaller sepals are oval in shape, measuring about 3–4 mm by 4–5 mm. The stamens vary in length from 1.7 to 2.5 mm. The flowering period typically occurs between April and June, with pollen released in tetrads. The plant produces compound fruits consisting of numerous fused, fleshy, and bumpy carpels, which are oval or round, measuring approximately 2.5–5 cm in length and 2.5–4 cm in width. Initially green, the fruit ripens to yellow and eventually turns orange, containing multiple oblong, cylindrical seeds of a burnt-orange color. The fruit stalk measures between 1.5–5 cm in length. A. senegalese is predominantly pollinated by beetles but can also be manually pollinated when cultivated as a crop. Its seeds have a viability period of about six months (AgroForestry Tree Database, 2013).

Figure 2. Annona senegalensis

Uses of Annona senegalensis

The primary use of this versatile plant is for food, but it has applications in numerous aspects of human endeavor, and every part of the plant has unique properties and uses. The flowers, leaves and fruit are edible and culinary: white fruit pulp has a mild, pineapple-like flavor (AgroForestry Tree Database, 2013). Flowers are added to spice or garnish meals; leaves are eaten by humans as vegetables, or grazed by livestock. Leaves are also part of the diet of the West African giraffe. The leaves are also used to create a general health tonic, in the treatment of pneumonia, and as mattress and pillow stuffing. Specific to Sudan, leaves are boiled in the making of perfume. Bark can be processed to produce yellow-brown dye, insecticide, or medicine for treating a wide array of ailments, including worms parasitic on the intestines or flesh (notably guinea worms), diarrhea, gastroenteritis, lung infections, toothaches, and even snakebites. Natural gum in the bark is used to close open wounds (AgroForestry Tree Database, 2013).

Roots are also used medicinally in treating a gamut of conditions, from dizziness and indigestion to chest colds to venereal diseases. Suckering shoots provide binding fibers, and the malleable, pale brown to white wood is used to carve tool handles, or fashioned into poles. Wood ash is an admixture to chewing tobacco and snuff, and also in soap production as solvent. The essential oils in the fruits and leaves are valued for their organic chemical constituents: car-3-ene (in fruit) and linalool (from leaves). Certain parts of A. senegalese are used in treating skin or eye disorders. Many South Africans believe the roots can cure insanity. Some Mozambicans feed them to infants to wean them from their mother's breast (AgroForestry Tree Database, 2013).

Alkaloids

Alkaloids are low-weight nitrogen-containing secondary metabolites, classified according to the carbon-nitrogen ring such as isoquinoline, pyridine, indole, piper-idine, and pyrrole. Aporphine alkaloids are found in majority of Annona species followed by derivatives of oxoapophine. About 46% of the total ALKs are concentrated in leaves, and only 3.5% of it is reported in roots and seeds (Egydio-Brandão et al., 2017).

Acetogenins

Acetogenins (ACGs) are naturally occurring compounds recognized as the primary bioactive constituents of the Annonaceae family (Durán et al., 2021). They are formed through the enzymatic combination of a three-carbon unit with lacceroic (C-32) and ghedoic (C-34) fatty acids. Structurally, ACGs consist of long-chain aliphatic fatty acids (C-35/C-37) that incorporate one or more tetrahydrofuran (THF) rings, double bonds, oxygenated functional groups such as ketones, epoxides, and hydroxyls, as well as a butyrolactone moiety at the end of the hydrocarbon chain.

Essential Oils

Terpenes and phenylpropenes are two essential components of complex mixture of EOs. Analysis of various parts of Annona plants mainly leaves has shown the presence of various oils, and the derivatives of sesquiterpenes are among the major Eos (Cascaes et al., 2021).

Biological Activities in Annona Senegalese

Antioxidant Activity.

Antimicrobial and Antifungal Activity.

Antileishmanial, Trypanocidal, and Antimalarial Activity.

Antitumor Activity.

Antidiabetic Activity.

Hepatoprotective Activity.

Antiulcer Activity.

Antidiarrheal Activity.

MATERIAL AND METHODS

Apparatus

Electric blender 9X1000 Newclime France), Micropipette (CE-IVD Lambmat India), spectrophotometer (UV 751 Shangahi Youke Instrument Co Ltd China), Water Bath (HH-W21-Cr42II India Mart India), Weighing Balance (PB 3002-5 Mettler Toledo Switzerland), DYMIND Haematological analyser (DYMIND DH76 Biotech, China)

Reagents

Diethyl nitrosamine (DEN)-Induced Hepatocellular Carcinoma, Silymarin Standard, EDTA Bottles, Ethanol

Plant Material

The leaves extract Annona senegalenses was obtained from Vicinity of federal university of Wukari

Preparation of Plant Extract

The collected plant materials were washed sliced and completely shade dry. The dried material was ground make to a fine powder and used for extraction. The powdered plant material Annona senegalese (200 g) was extracted with ethanol (1 litre) in an air tight clean flat-bottomed container for 48 hours at room temperature with occasional stirring and shaking (Trease and Evans, 2002). The ethanol extract was filtered first through a fresh cotton plug and then through a what man filter. The filtrate was evaporated to dryness in vacuo by a rotary evaporator at 40-50°C and the extract was kept in a well tight sterile bottle/container under refrigerated conditions until use.

Animals

Thirty-six (36) male albino rats (weighing between 140 ±20 g) were obtained from HAUEMM Veterinary Animal House, Federal Housing Estate. Adamawa state, Nigeria. They were housed in polypropylene cages, and were given standard grower diet (Vital Feeds, Jos) and water ad labium for 7 days to enable them acclimatize before the commencement of the experiment. Throughout the experiment it was maintained under the laboratory conditions of 29 ± 2°C (temperature) and 12 hours light and dark cycle in the Department of Biochemistry, Federal University Wukari, Taraba, Nigeria. Guide for the Care and Use of Laboratory Animals was strictly followed.

Experimental Design

The rats will be randomly divided into six equal groups of six rats each. Group I served as normal control, that is, no inducement and no administration of ethanol extract as shown in the group treatment below. Liver carcinogenesis was induced in group II, III, IV V, and VI, by injecting diethyl nitrosamine (in DMSO) intraperitonially at a dose of 50 mg/kg body weight once in a week for a period of three weeks as reported by (Sumithra et al., 2013). Group II served as the negative control while group III served as the positive

control group (silymarin 100 mg/kg body weight was used as standard drug). The ethanol extract of the plant extract was administered to group IV (200 mg/kg b.w.) and group V (400 mg/kg b,w.) and group VI (600 mg/kg b,w.) respectively. The ethanol extract, will be administered to the rats through oral gavages for a period of 14 days.

Group Treatment

I (Normal control) Normal + No treatment

II (Negative control) DEN + No treatment

III (Positive Control) DEN + Standard Drug (Silymarin 100 mg/kg bw)

IV (Treatment I) DEN + Methanol Extract (200 mg/kg b.w)

V (Treatment II) DEN + Methanol Extract (400 mg/kg b.w)

VI (Treatment III) DEN + Methanol Extract (600 mg/kg b.w)

Collection of Samples

On completion of the experimental period, animals were anaesthetized with diethyl either (2ml/kg). The blood was collected with and without EDTA as anticoagulant.

Statistical Analysis

The mean ± SD of all values was calculated and changes observed between the treatment group and the control were subjected to analysis of variance (ANOVA) using -SPSS version 23

RESULTS

Kidney Functions

Table 1: Effect of Leaves extract of Annona senegalensis on kidney functions

Parameter	Normal No Induction, No Treatment	Negative DEN+No Treatment	Positive DEN + 100mg/Kg Of Silymarin	DEN+200mg/Kg Bwt. of the Extract	DEN +400mg/Kg Bwt. of the Extract	DEN+600mg/Kg Bwt. Of the Extract
UREA	18.25 ± 0.96^{c}	34.53 ± 0.70^{d}	$17.0.8361\pm0.93^{b,c}$	16.45 ± 1.03^{b}	16.18 ± 0.83^{b}	14.35 ± 0.77^{a}
CREATENIE	$1.8\pm0.16^{a,b}$	3.72 ± 0.5^{d}	1.5 ± 0.32^{a}	2.51 ± 0.08^{c}	$2.18\pm0.21^{b,c}$	1.44 ± 0.10^{a}

Parameter	Normal No Induction, No Treatment	Negative DEN+No Treatment	Positive DEN + 100mg/Kg Of Silymarin	DEN+200mg/Kg Bwt. of the Extract	DEN +400mg/Kg Bwt. of the Extract	DEN+600mg/Kg Bwt. Of the Extract
SODIUM	204.43 ± 1.03^{a}	498.07 ± 1.5^{f}	233.83 ± 1.4^{b}	245.4 ± 0.10^{c}	257.5 ± 0.10^{d}	270.4 ± 0.7^{e}
POTASSIUM	2.7 ± 0.10^{b}	3.7 ± 0.6^{c}	1.9 ± 0.23^{a}	6.35 ± 0.5^{e}	4.62 ± 0.30^{d}	3.90 ± 0.08^{c}
CHLORIDE	60.8 ± 0.7^{a}	160.10 ± 0.10^{d}	81.11 ± 0.92^{b}	120.8 ± 0.63^{c}	80.53 ± 1.11^{b}	81.31 ± 0.82^{b}
CO_2	51.51 ± 1.2^{a}	115.32 ± 0.80^{e}	51.50 ± 1.14^{a}	81.53 ± 0.80^{c}	88.60 ± 0.10^{d}	77.6 ± 1.13^{b}

The values were presented as mean ± S.E.M (n=3). Values not sharing the same superscript letters are significantly different ($p<0.05$). Values with the same superscript are not significantly different ($p<0.05$).

Urea Levels: In healthy individuals, urea levels are usually in the normal range. However, in the group with no treatment, urea levels were significantly higher, suggesting potential kidney issues. Fortunately, when the Leaves extract was given in doses of 400 mg/kg and 600 mg/kg, the urea levels improved, indicating that the extract may help support kidney health.

Creatinine Levels: Similar to urea, creatinine levels were alarmingly high in the untreated group, which can signal kidney dysfunction. The extract appeared to help lower creatinine levels, especially at the higher doses (400 and 600mg/kg), indicating that it may protect the kidneys and promote better function.

Sodium Levels: High sodium levels can indicate that the kidneys are struggling to filter properly. The untreated group showed elevated sodium levels, but the extract helped reduce these levels at higher doses, suggesting a positive impact on kidney function.

Potassium Levels: Elevated potassium is another sign that kidneys may not be functioning well. The extract significantly reduced potassium levels, particularly at the higher doses (400 mg/kg and 600 mg/kg), indicating it might help restore normal kidney function.

Chloride Levels: The chloride levels were elevated in the untreated group, but they improved with the extract, showing that it can help maintain a healthy balance of electrolytes in the body.

CO2 Levels: High CO2 levels can indicate metabolic problems or kidney issues. In the untreated group, CO2 was significantly high, but those given the extract had lower levels of (200 mg/kg) suggesting that it may help restore acid-base balance.

Lipid Profile

Table 2: Effect of Leaves extract of Annona senegalensis on lipid profile

PARAMETER	NORMAL	NEGATIVE DEN + No treatment	POSITIVE DEN +Standard Drug (Silymarin 100 mg/kg bwt.)	DEN + 200mg/kg bwt.) OF THE EXTRACT	DEN + 400mg/kg bwt.) OF THE EXTRACT	DEN + 600mg/kg bwt.) OF THE EXTRACT
CHOLESTEROL	151.36±1.10^{a}	368.56±1.71^{e}	151.31±1.07^{a}	264.03±5.13^{d}	217.46±0.82^{c}	182.24±1.2^{b}
TRIGLYCERIDE	176.04±0.90^{a}	401.13±1.10^{e}	201.31±1.05^{b}	336.29±1.12^{d}	328.91±12.04^{d}	318.99±1.31^{c}
HDL	12.46±0.62^{b}	9.30±0.92^{a}	14.84±1.3^{c}	15.77±0.71^{c}	15.64±1.06^{c}	15.94±1.6^{c}
LDL	102.74±0.60^{b}	276.68±1.10^{e}	95.85±1.21^{a}	182.31±1.60^{d}	134.87±1.40^{c}	103.47±0.73^{b}

The values were presented as mean ± S.E.M (n=3). Values not sharing the same superscript letters are significantly different ($p<0.05$). Values with the same superscript are not significantly different ($p<0.05$).

Cholesterol levels are significantly elevated in the negative group, indicating dyslipidemia. The extract doses show a reduction in cholesterol levels, particularly at 400mg/kg and 600mg/kg, suggesting a beneficial effect on lipid metabolism.

Triglycerides are also high in the negative group, reflecting poor lipid regulation. The extract doses show varying results, with the 200 mg/kg dose maintaining high triglyceride levels but lower levels observed at 400 mg/kg and 600 mg/kg, indicating some improvement.

HDL (High-Density Lipoprotein) is lower in the negative group compared to normal levels. The extract shows increase in HDL at higher doses, particularly at 400 mg/kg and 600 mg/kg, suggesting a potential protective effect against cardiovascular risk.

LDL (Low-Density Lipoprotein) levels are significantly elevated in the negative group. The extract reduces LDL levels notably at lower doses (200 mg/kg), with a less pronounced effect at higher dosages, indicating potential benefits in improving lipid profiles.

Overall, the leaves extract of Annona senegalensis appears to have a positive effect on lipid profiles by reducing cholesterol and LDL levels while increasing HDL levels, particularly at higher doses. This suggests its potential role in improving lipid metabolism and reducing cardiovascular risk.

DISCUSSION

The presence of tannins, alkaloids, saponins, sterols, triterpenes and reducing sugar in Annona senegalensisi gives it the ability to suppress and revers hepatorenal disorders. As reported by Ahmad et al., 2020 in his study titled "Evaluation of the Antidiarrhoeal Activity of Aqueous Root and Stem Bark Extracts of Annona senegalensis" that the presence of these phytochemicals in A. senegalensis root and stem barks may be responsible for its antidiarrhoeal activity. However, in this study, the leave extracts of Annona senegalensis demonstrated a promising hepatorenal protectivity as it can be seen in Urea Levels in the group with no treatment, urea levels were significantly higher (34.53±0.70), suggesting potential kidney issues. Fortunately, when the Leaves extract was given in doses of 200 mg/kg and 400 mg/kg, the urea levels improved (16.45±1.03 and 16.18±0.83 respectively), indicating that the extract can help support kidney health. Again, Creatinine levels follow similar trend, creatinine levels were alarmingly high in the untreated group going as high as (3.72±0.5), which can signal kidney dysfunction. The extract appeared to help lower creatinine levels, especially at the higher doses (600mg/kg), indicating that it may protect the kidneys and promote better function. The ethanol extract demonstrated promising activity in electrolyte balance as 264.03±5.13it can be seen in Sodium, potassium, and chloride ion levels, high electrolyte levels can indicate that the kidneys are struggling to filter properly. The untreated group showed elevated electrolyte levels, but the extract helped reduce these levels at higher doses, showing that it can help maintain a healthy balance of electrolytes in the body.

Adversely, the leave extract of Annona senegalensis appears to have a positive effect on lipid profiles by reducing cholesterol from 368.57±1.72 in the negative control

group to 266.40±0.88 and 217.46±0.82 and 182.24±1.2 in both 200, 400, and 600mg/kg of the ethanolic extract, and at same veil maintaining good HDL levels, particularly at higher doses. This suggests its potential role in improving lipid metabolism and reducing cardiovascular risk.

CONCLUSION

This study concludes that the plant extract (Annona senegalensis) can considerably raise blood lipid levels and renal function. the extract helped lower waste products like urea and creatinine and balanced electrolytes when given at doses of 200 mg/kg and 400 mg/kg, more likely 600mg/kg indicating healthier kidneys. This suggests that the plant extract may shield the kidneys from harm and promote more regular kidney function.

Additionally, the extract improved lipid metabolism at the same time. It increases good HDL levels and decreased bad LDL and cholesterol, which may minimize the risk of heart disease.

Although encouraging, more research is required to completely understand how this natural molecule functions and its potential as a therapeutic agent. Overall, these data point to Annona senegalensis helping kidney health and fostering a healthier lipid profile.

Recommendations

It is recommended that:

1. Annona senegalensis extract shows promise for improving kidney function and lipid profiles. Therefore, it's recommended that healthcare providers consider incorporating Annona senegalensis into treatment plans for patients with kidney or lipid disorders, using dosages of 200-600mg/kg.

2. Regular monitoring of relevant health markers is crucial to ensure safety and effectiveness. Further research, including clinical trials, is needed to confirm these findings and understand the mechanisms of action.

3. Finally, patients should combine Annona senegalensis treatment with healthy lifestyle changes, such as a balanced diet and exercise, to maximize benefits.

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