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Effect of Methanol Stem Bark Extract of *Annona senegalensis* on Haematological Profile in Diethylnitrosamine-Induced Hepatocellular Carcinoma in Rats

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Abstract

Liver cancer, particularly hepatocellular carcinoma (HCC), is a leading cause of mortality worldwide and is frequently associated with hematological complications such as anemia and impaired blood clotting. This study evaluated the hematoprotective effects of methanol stem-bark extract of *Annona senegalensis* in rats with diethylnitrosamine (DEN)-induced HCC. Fifty-four male rats were allocated into six groups: a normal control group, a DEN-induced group without treatment, a positive control group treated with silymarin, and three treatment groups administered varying doses of *A. senegalensis* extract. Hematological parameters assessed included red blood cells (RBC), hemoglobin (HGB), white blood cells (WBC), hematocrit (HCT), and platelets (PLT). DEN induction significantly reduced RBC, HGB, and PLT levels, indicating anemia and coagulation impairment. Treatment with *A. senegalensis*, particularly at 400 mg/kg, markedly improved these hematological

indices, suggesting a restorative effect on blood parameters. The protective role of the extract may be attributed to its bioactive constituents, such as flavonoids, alkaloids, and tannins, known for their antioxidant and anti-inflammatory activities. These findings suggest that *A. senegalensis* possesses promising hematoprotective potential in the management of blood-related complications associated with liver cancer. Further research, including mechanistic studies and clinical trials, is warranted to validate its safety and therapeutic efficacy in humans.

Keywords: Methanol; Stem Bark Extract; *Annona senegalensis*; Hematological Parameters; Diethylnitrosamine; Hepatocellular Carcinoma; Wistar Rats

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-associated deaths, and globally it is three times more common in men than women (Siegel *et al.,* 2017). Hepatocellular Carcinoma is one of the leading causes of cancer related deaths in the Kingdom of Saudi Arabia (Abusanad et al., 2022). Hepatocellular Carcinoma is the 4th most common cancer in males and 8thcommon cancer in females accounting for 4.3% of the reported cancer cases in the year 2013 (Acharya, 2014). A global estimation of 40,710 new cases and 28,920 deaths due to liver and intrahepatic bile duct cancers in 2017 highlights the need for improvement in current management strategies (Khan et al., 2017). Common risk factors for liver cancer include heavy alcohol consumption, hepatitis B virus (HBV), hepatitis C virus, obesity, tobacco smoking, diabetes and genetic factors. Partial hepatectomy, embolization and sorafenib (Nexavar®) offer partial to complete success depending upon the stage of the disease (Lurje et al., 2019). The incidence of high mortality and associated side effects following chemotherapy and/or radiotherapy increase the demand for alternative medicine for cancer treatment (Johnson et al., 2018). Not surprisingly, many potent anticancer compounds were isolated from the plants viz. doxorubicin, taxol, etoposide, cisplatin, vinblastine, vindesine, vincristine and topotecan (Malabadi et al., 2024). Given the current interests in alternative medicine, it is imperative that much-concerted effects are undertaken to identify novel compounds that will aid therapy and also help to avoid its unjustifiable use.

Diethylnitrosamine, a potent hepatocarcinogen, has been widely used in experimental studies to induce liver cancer in animal models, particularly in rats (Schulien

and Hasselblatt, 2022). Upon administration, DEN undergoes metabolic activation by cytochrome P450 enzymes, forming reactive intermediates that can cause DNA damage and initiate carcinogenesis (Li and Hecht, 2022). This model provides a valuable tool for investigating potential chemo preventive agents against HCC.

Natural products, particularly plant extracts, have gained attention as potential chemo preventive agents due to their diverse biological activities, including antioxidant, anti-inflammatory, and anticancer properties (Yi et al., 2017). Annona senegalensis, commonly known as African custard apple, is a medicinal plant traditionally used in various African cultures for its therapeutic effects (Tukur et al., 2020). The stem bark of Annona senegalensis is rich in phytochemicals, including alkaloids, flavonoids, and tannins, which have been shown to exhibit antifungal, antibacterial, and anticancer activities (Diallo et al., 2024).

Recent studies have highlighted the importance of exploring plant-based extracts for their dual benefits: combating infections and mitigating cancer risk. Fungal infections can complicate the treatment of cancer patients, leading to increased morbidity and mortality (Ruhnke et al., 2020). Therefore, the antifungal properties of Annona senegalensis could provide an additional therapeutic advantage in the context of HCC treatment (Attanayake et al., 2024). The method of methanol extraction is particularly effective in isolating bioactive compounds, making it a suitable choice for evaluating the medicinal properties of this plant.

The haematological profile is an essential aspect of assessing the overall health and physiological status of an organism, especially in models of disease (Chen and Luo, 2023). Alterations in blood parameters can indicate the presence of disease or the effects of treatment. Changes in haematological parameters, such as red blood cell count, white blood cell count, and hemoglobin levels, can provide insights into the systemic effects of the disease and the potential protective role of phytotherapy.

Many in vitro and in vivo models of hepatic cancer have been utilized in the past to evaluate various drugs (Xing et al., 2017). In vivo hepatic cancer model development is done using various chemical methods, of which diethylnitrosamine (DEN) is a chemical carcinogen, commonly utilized to induce liver carcinogenesis by increasing oxidative stress and liver damage (Schulien and Hasselblatt, 2021). Hence in the present study, we used the DEN-induced hepatic cancer model in Wistar rats and evaluated the cancer inhibition

effects of the ethanol stem-bark extracts of *Annona senegalensis* on haematological parameters and its Anti-fungal activities.

Statement of problem

Hepatocellular carcinoma (HCC) is one of the most common and aggressive forms of liver cancer, with limited treatment options and poor prognosis in advanced stages. Diethylnitrosamine (DEN) is a well-known hepatocarcinogen that induces HCC in experimental models, mimicking the pathological and molecular features of human liver cancer. Current therapeutic strategies for HCC, such as chemotherapy and surgery, are often associated with significant side effects and limited efficacy, highlighting the need for alternative and complementary treatments.

Justification of study

Hepatocellular carcinoma is one of the most common and deadly cancers worldwide, particularly in regions with high rates of hepatitis B and C infections. Despite advances in medical science, the prognosis for HCC remains poor, especially in advanced stages, and existing therapies such as chemotherapy, radiation, and surgery are often associated with severe side effects, high costs, and limited accessibility. This underscores the urgent need for alternative and complementary treatments that are effective, affordable, and minimally toxic.

Aim

The aim of this study is to evaluate the haematological indices of effects of methanol stembark extracts of *Annona senegalensis* on N-Nitrosodiethylamine induced hepatocarcinogenesis on wistar rats.

Objectives

1. Administration of methanol stembark extracts of *Anonna senegalensis* on N-Nitrosodiethylamine induced hepatocarcinogenesis on wistar rats.

2. To determine the levels of haematological indices methanol stembark extracts of *Anonna senegalensis* on N-Nitrosodiethylamine induced hepatocarcinogenesis on wistar rats

Scope of the study

This study focuses on evaluating the haematoprotective effects of methanol stem bark extract of *Annona senegalensis* in Wistar rats induced with hepatocellular carcinoma

using diethylnitrosamine (DEN). The research investigates key haematological parameters, including red blood cell (RBC) count, haemoglobin (HGB) concentration, white blood cell (WBC) count, haematocrit (HCT), and platelet (PLT) levels. The study is limited to an experimental animal model and does not extend to human trials. It aims to provide preliminary data on the potential benefits of *Annona senegalensis* in liver-related haematological disorders, paving the way for further toxicological evaluations and clinical studies.

Literature review

Botanical description *Annona Senegalese*

Morphology:

Tree Characteristics*: Annona senegalensis*, commonly known as the African custard apple or wild custard apple, is a small deciduous shrub or tree that typically grows to a height of 2 to 6 meters. The tree has a rough, greyish-brown bark with a corky texture, and its branches often spread widely, forming a dense canopy. The inner bark is yellowish and fibrous, which is characteristic of many medicinal plants in the Annonaceae family (Okwu, 2004; Akaneme, 2008).

Leaves: The leaves of *Annona senegalensis* are simple, alternate, and oblong to elliptical in shape, measuring about 5 to 15 cm in length and 2 to 6 cm in width. The leaf margins are entire, and the upper surface is dark green, while the underside is lighter with a slightly hairy texture. The leaves are aromatic when crushed, a feature that contributes to their use in traditional medicine (Burits and Bucar, 2002; Adebayo *et al.,* 2010).

Flowers: The flowers of *Annona senegalensis* are solitary or occur in small clusters. They are typically yellowish-green or cream-colored, with three outer petals and three smaller inner petals. The flowers are fragrant and attract various pollinators, including beetles and bees. The flowering period usually occurs during the rainy season, depending on the geographical location (Idowu *et al.,* 2006; Okwu and Emenike, 2006).

Fruits: The fruit of *Annona senegalensis* is a fleshy, aggregate berry, often referred to as a "custard apple."It is spherical or slightly oval, measuring about 2 to 5 cm in diameter. The fruit 's skin is green when unripe, turning yellowish or orange when ripe. The pulp is

soft, sweet, and edible, containing numerous black seeds embedded within. The seeds are glossy, flattened, and have a hard-outer coat (Asare *et al.,* 2015; Ukana *et al.,* 2012).

Habitat and Distribution:

Annona senegalensis is native to tropical and subtropical regions of Africa, particularly in savannahs, woodlands, and dry forests. It thrives in well-drained soils and is commonly found in countries such as Nigeria, Senegal, Sudan, Uganda, and Tanzania. The plant is highly adaptable and can grow in arid and semi-arid environments, making it a resilient species in various ecological zones (Amadi *et al.,* 2000; Ordinioha and Brisibe, 2013).

Traditional Uses:

Annona senegalensis has been widely used in traditional African medicine for centuries. Various parts of the plant, including the bark, leaves, roots, and fruits, are utilized for their medicinal properties. The stem bark is known for its antimicrobial, anti-inflammatory, and hepatoprotective effects. In some cultures, bark is boiled and used as a remedy for gastrointestinal disorders, fever, and skin infections. The leaves are often applied as poultices to treat wounds, while the fruit is consumed for its nutritional value and as a natural remedy for digestive issues (Dede *et al.,* 2002; Patrick-Iwuanyanwu *et al.,* 2011).

Figure 1: *Annona Senegalese*

Phytochemical Constituents of *Annona Senegalese*

Annona senegalensis is a rich source of diverse phytochemicals, which contribute to its medicinal and nutritional properties. These bioactive compounds play a significant role in the plant 's therapeutic effects, including its antimicrobial, antioxidant, and anticancer activities. Below is a detailed discussion of the major phytochemical constituents found in Annona senegalensis

Alkaloids

Alkaloids are one of the most important classes of phytochemicals found in Annona senegalensis. These nitrogen-containing compounds are known for their potent biological activities, including antimicrobial, anti-inflammatory, and anticancer properties. Alkaloids such as annonaine and asimilobine have been isolated from the stem bark and leaves of *Annona senegalensis* and are believed to contribute to its medicinal effects (Okwu, 2004; Adebayo *et al.,* 2010). These compounds have been shown to inhibit the growth of pathogenic microorganisms and exhibit cytotoxic effects on cancer cells, making them valuable in traditional and modern medicine.

Flavonoids

Flavonoids are another major group of phytochemicals present in *Annona senegalensis*. These polyphenolic compounds are known for their strong antioxidant properties, which help neutralize free radicals and reduce oxidative stress. Flavonoids such as quercetin, kaempferol, and rutin have been identified in the leaves and stem bark of *Annona senegalensis* (Asare *et al.,* 2015; Wang *et al.,* 2011). These compounds play a crucial role in protecting cells from oxidative damage, which is a key factor in the development of chronic diseases such as cancer and cardiovascular disorders. Additionally, flavonoids exhibit anti-inflammatory and antimicrobial activities, further enhancing the plant 's therapeutic potential.

Tannins

Tannins are polyphenolic compounds that are widely distributed in Annona senegalensis, particularly in the bark and leaves. These compounds are known for their astringent properties and ability to bind and precipitate proteins. Tannins have been reported to exhibit antimicrobial, anti-inflammatory, and wound-healing properties (Okwu and Omodamiro, 2005; Adebayo *et al.,* 2011). In traditional medicine, tannins are often

used to treat gastrointestinal disorders, skin infections, and wounds due to their ability to reduce inflammation and promote tissue regeneration.

Saponins

Saponins are glycosides found in *Annona senegalensis* that are known for their foaming properties and diverse biological activities. These compounds have been shown to exhibit antimicrobial, anti-inflammatory, and anticancer effects (Akaneme, 2008; Idowu *et al.,* 2006). Saponins work by disrupting the cell membranes of microorganisms and cancer cells, leading to their destruction. Additionally, saponins have been reported to enhance immune function and reduce cholesterol levels, making them valuable in the management of various health conditions.

Phenolic Compounds

Phenolic compounds are abundant in *Annona senegalensis* and are responsible for its strong antioxidant activity. These compounds, including phenolic acids and flavonoids, help scavenge free radicals and protect cells from oxidative damage (Asare *et al.,* 2015; Wang *et al.,* 2011). The high phenolic content in Annona senegalensis contributes to its ability to mitigate oxidative stress, which is a key factor in the development of liver damage, cancer, and other chronic diseases.

Terpenoids

Terpenoids are a diverse group of phytochemicals found in *Annona senegalensis* that are known for their aromatic properties and wide range of biological activities. These compounds have been shown to exhibit antimicrobial, anti-inflammatory, and anticancer effects (Burits and Bucar, 2002; Okwu and Emenike, 2006). Terpenoids such as limonene and β-caryophyllene have been identified in *Annona senegalensis* and are believed to contribute to its therapeutic properties.

Pharmacological Studies on *Annona Senegalese*

Annona senegalensis has been extensively studied for its pharmacological properties, which include antimicrobial, anticancer, anti-inflammatory, and hepatoprotective effects. These activities are largely attributed to the plant 's rich phytochemical composition, including alkaloids, flavonoids, tannins, and terpenoids. Below is a detailed discussion of the pharmacological studies conducted on Annona senegalensis:

Antimicrobial Activities

Annona senegalensis has demonstrated significant antimicrobial properties against a wide range of pathogens, including bacteria, fungi, and parasites. The plant 's antimicrobial activity is primarily due to the presence of alkaloids, flavonoids, and tannins, which disrupt microbial cell walls, inhibit nucleic acid synthesis, and interfere with cell metabolism (Okwu and Morah, 2007; Burits and Bucar, 2002). Studies have shown that the methanolic extract of *Annona senegalensis* stem bark exhibits potent antibacterial activity against both Gram-positive and Gram-negative bacteria, including Staphylococcus aureus and Escherichia coli (Idowu *et al.,* 2003). Additionally, the plant has been traditionally used to treat infections such as malaria, skin conditions, and gastrointestinal disorders, further validating its antimicrobial potential (Akaneme, 2008).

Anticancer and Cellular Protective Actions

The anticancer properties of *Annona senegalensis* are attributed to its diverse phytochemical constituents, including flavonoids, tannins, and alkaloids. These compounds play a crucial role in combating oxidative stress, which is a key factor in cancer development and cellular damage. The antioxidant properties of Annona senegalensis, primarily driven by its high phenolic and flavonoid content, effectively neutralize reactive oxygen species (ROS), preventing oxidative damage to DNA and other cellular components (Olorunnisola *et al.,* 2008; Asare *et al.,* 2015). Flavonoids such as quercetin and kaempferol have been shown to induce apoptosis (programmed cell death) in cancer cells, inhibit cell proliferation, and suppress angiogenesis, which is the process that allows tumors to grow their own blood vessels (Burits and Bucar, 2002). These actions not only help in reducing tumor growth but also prevent cancer cells from spreading to other tissues.

Anti-inflammatory and Analgesic Properties

Annona senegalensis has been recognized for its significant anti-inflammatory and analgesic (pain-relieving) properties. These effects are largely attributed to the presence of flavonoids, tannins, and alkaloids, which modulate inflammatory pathways and reduce the production of pro-inflammatory cytokines (Okwu and Emenike, 2006). The plant 's anti-inflammatory activity has been demonstrated in various studies, where extracts of *Annona senegalensis* were shown to inhibit key enzymes involved in the inflammatory response, such as cyclooxygenase (COX) and lipoxygenase (LOX) (Idowu *et al.,* 2006). Additionally, the

plant has been traditionally used to manage conditions such as arthritis, muscle pain, and fever, further supporting its role as a natural anti-inflammatory agent.

Hepatoprotective Effects

The hepatoprotective properties of *Annona senegalensis* have been widely studied, particularly in models of liver damage induced by toxins such as carbon tetrachloride (CCl_4) and acetaminophen. The plant 's ability to protect the liver is attributed to its antioxidant and anti-inflammatory properties, which help reduce oxidative stress and inflammation in hepatic tissues (Adebayo *et al.,* 2011; Adeoye *et al.,* 2020). Studies have shown that *Annona senegalensis* extract significantly reduces serum levels of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), which are elevated during liver damage. Additionally, the plant enhances the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), further protecting liver cells from oxidative damage (Patrick-Iwuanyanwu *et al.,* 2011).

Mechanism of Action of *Annona Senegalese* on Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is one of the most common and aggressive forms of liver cancer, often associated with chronic liver damage, oxidative stress, and inflammation. *Annona senegalensis* has been studied for its potential to mitigate HCC through various mechanisms, including its antioxidant, anti-inflammatory, and antiproliferative properties. Below is a detailed discussion of the mechanisms by which *Annona senegalensis* exerts its effects on hepatocellular carcinoma:

Antioxidant Activity and Oxidative Stress Reduction

Oxidative stress plays a critical role in the development and progression of hepatocellular carcinoma. Reactive oxygen species (ROS) generated during chronic liver damage can cause DNA damage, lipid peroxidation, and protein oxidation, leading to the initiation and progression of cancer. *Annona senegalensis* contains high levels of phenolic compounds, flavonoids, and alkaloids, which exhibit strong antioxidant properties (Asare *et al.,* 2015; Wang *et al.,* 2011). These compounds neutralize ROS, reduce oxidative stress, and protect liver cells from damage. Studies have shown that *Annona senegalensis* extract significantly increases the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which are crucial for

maintaining cellular redox balance (Adebayo *et al.,* 2011). By enhancing antioxidant defenses, *Annona senegalensis* helps prevent the oxidative damage that contributes to the development of HCC.

Anti-inflammatory Effects

Chronic inflammation is a key driver of hepatocellular carcinoma, as it promotes cell proliferation, survival, and angiogenesis. *Annona senegalensis* has been shown to exhibit potent anti-inflammatory effects, primarily through the inhibition of pro-inflammatory signaling pathways. Flavonoids and tannins in the plant inhibit the activation of nuclear factor kappa B (NF-ϰB), a transcription factor that regulates the expression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Okwu and Emenike, 2006; Idowu *et al.,* 2006). By downregulating NF-ϰB and reducing the production of inflammatory mediators, *Annona senegalensis* helps suppress the inflammatory environment that supports tumor growth and progression.

Induction of Apoptosis in Cancer Cells

Apoptosis, or programmed cell death, is a critical mechanism for eliminating damaged or cancerous cells. *Annona senegalensis* has been shown to induce apoptosis in hepatocellular carcinoma cells through the activation of intrinsic and extrinsic apoptotic pathways. Flavonoids such as quercetin and kaempferol, present in Annona senegalensis, have been reported to upregulate pro-apoptotic proteins such as Bax and downregulate anti-apoptotic proteins such as Bcl-2 (Burits and Bucar, 2002). Additionally, the plant 's alkaloids have been shown to activate caspase enzymes, which play a central role in the execution of apoptosis (Okwu, 2004). By inducing apoptosis in cancer cells, Annona senegalensi helps reduce tumor growth and prevent the spread of cancer to other tissues.

Inhibition of Angiogenesis

Angiogenesis, the formation of new blood vessels, is essential for tumor growth and metastasis. *Annona senegalensis* has been shown to inhibit angiogenesis by suppressing the expression of vascular endothelial growth factor (VEGF), a key mediator of blood vessel formation (Akaneme, 2008). Flavonoids and tannins in the plant interfere with the signaling pathways that promote angiogenesis, thereby limiting the blood supply to tumors and inhibiting their growth. This anti-angiogenic effect is particularly important in hepatocellular carcinoma, where tumor progression is highly dependent on the formation of new blood vessels.

Modulation of Cell Cycle and Proliferation

Annona senegalensis has been shown to inhibit the proliferation of hepatocellular carcinoma cells by inducing cell cycle arrest. The plant 's bioactive compounds, particularly flavonoids and alkaloids, interfere with the cell cycle at various checkpoints, preventing the uncontrolled division of cancer cells (Olorunnisola *et al.,* 2008). For example, studies have shown that *Annona senegalensis* extract induces G1/S phase arrest in cancer cells, thereby inhibiting their proliferation and promoting cell death (Patrick-Iwuanyanwu *et al.,* 2011). This antiproliferative effect is crucial for controlling tumor growth and preventing the spread of cancer.

Enhancement of Immune Surveillance

The immune system plays a critical role in recognizing and eliminating cancer cells. *Annona senegalensis* has been shown to enhance immune surveillance by stimulating the activity of immune cells such as natural killer (NK) cells and macrophages (Adebayo *et al.,* 2010). Saponins and flavonoids in the plant enhance the immune system 's ability to detect and destroy cancer cells, thereby reducing tumor growth and metastasis. This immunomodulatory effect is particularly important in hepatocellular carcinoma, where immune evasion is a common feature of tumor progression.

Protection Against Diethylnitrosamine (DEN)-Induced Liver Damage

Diethylnitrosamine (DEN) is a potent hepatocarcinogen that induces liver damage and hepatocellular carcinoma in animal models. *Annona senegalensis* has been shown to protect against DEN-induced liver damage by reducing oxidative stress, inflammation, and DNA damage (Choumessi *et al.,* 2012). The plant 's antioxidant and anti-inflammatory properties help mitigate the toxic effects of DEN, thereby preventing the initiation and progression of hepatocellular carcinoma. Additionally, *Annona senegalensis* enhances the liver 's detoxification capacity, further protecting against carcinogen-induced damage.

Overview of Diethyl nitrosamine (DEN)

Diethylnitrosamine (DEN) is a nitrosamine compound that is highly carcinogenic to the liver. It is commonly used in laboratory studies to induce hepatocellular carcinoma in animal models, particularly rats and mice. DEN is metabolized in the liver by cytochrome P450 enzymes, leading to the formation of reactive intermediates that cause DNA damage, oxidative stress, and inflammation (Choumessi *et al.,* 2012). These processes initiate and

promote the development of liver tumors, making DEN a reliable tool for studying liver carcinogenesis.

Mechanism of DEN-Induced Liver Damage

The carcinogenic effects of DEN are primarily mediated through its metabolic activation in the liver. Upon administration, DEN is metabolized by cytochrome P450 enzymes, particularly CYP2E1, to form reactive intermediates such as ethyldiazonium ions and carbon-centered radicals (Odo *et al.,* 2012). These reactive species alkylate DNA, proteins, and lipids, leading to the following key events:

DNA Damage: The alkylation of DNA by DEN metabolites results in the formation of DNA adducts, which can cause mutations in critical genes such as p53 and ras. These mutations disrupt normal cell cycle regulation and promote uncontrolled cell proliferation, a hallmark of cancer (Deplege, 2002).

Oxidative Stress: DEN metabolism generates reactive oxygen species (ROS), which cause oxidative damage to cellular components, including lipids, proteins, and DNA. This oxidative stress overwhelms the liver 's antioxidant defenses, leading to lipid peroxidation, protein oxidation, and DNA damage (Choumessi *et al.,* 2012).

Inflammation: DEN-induced liver damage triggers an inflammatory response characterized by the activation of Kupffer cells (liver macrophages) and the release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). Chronic inflammation promotes cell survival, proliferation, and angiogenesis, creating a microenvironment conducive to tumor development (Ordinioha and Brisibe, 2013).

Stages of DEN-Induced Hepatocellular Carcinoma

DEN-induced hepatocellular carcinoma typically progresses through several stages, including initiation, promotion, and progression:

Initiation: During the initiation phase, DEN metabolites cause DNA damage and mutations in hepatocytes, leading to the formation of initiated cells with malignant potential. These cells are characterized by genetic and epigenetic alterations that disrupt normal cellular functions (Amadi *et al.,* 2000).

Promotion: The promotion phase involves the clonal expansion of initiated cells, driven by oxidative stress, inflammation, and the activation of signaling pathways such as

NF-ϰB and STAT3. These pathways promote cell survival, proliferation, and resistance to apoptosis, facilitating the growth of preneoplastic lesions (Patrick-Iwuanyanwu *et al.,* 2011).

Progression: In the progression phase, preneoplastic lesions develop into fully malignant tumors. This stage is characterized by increased genomic instability, angiogenesis, and metastasis. DEN-induced tumors often exhibit features similar to human HCC, including high proliferation rates, vascular invasion, and poor differentiation (Onwurah and Eze, 2000).

Role of Oxidative Stress in DEN-Induced HCC

Oxidative stress plays a central role in DEN-induced hepatocellular carcinoma. The generation of ROS during DEN metabolism leads to the oxidation of cellular components, including DNA, lipids, and proteins. This oxidative damage contributes to the following processes:

DNA Mutations: ROS-induced DNA damage results in mutations in oncogenes and tumor suppressor genes, promoting the initiation and progression of HCC (Deplege, 2002).

Lipid Peroxidation: ROS-mediated lipid peroxidation disrupts cell membrane integrity and function, leading to cell death or the release of pro-inflammatory mediators that promote tumor growth (Odo *et al.,* 2012).

Protein Oxidation: The oxidation of proteins involved in cell cycle regulation, DNA repair, and apoptosis further contributes to the malignant transformation of hepatocytes (Choumessi *et al.,* 2012).

Inflammation and DEN-Induced HCC

Chronic inflammation is a key driver of DEN-induced hepatocellular carcinoma. The inflammatory response triggered by DEN-induced liver damage involves the activation of Kupffer cells, the release of pro-inflammatory cytokines, and the recruitment of immune cells to the liver. These processes create a tumor-promoting microenvironment characterized by:

Cytokine Production: Pro-inflammatory cytokines such as TNF-α and IL-6 promote cell survival, proliferation, and angiogenesis, facilitating tumor growth (Ordinioha and Brisibe, 2013).

NF-ϰB Activation: The activation of NF-ϰB, a transcription factor that regulates the expression of pro-inflammatory genes, plays a critical role in DEN-induced HCC. NF-

κB promotes the survival of initiated cells and the progression of preneoplastic lesions to malignant tumors (Patrick-Iwuanyanwu *et al.,* 2011).

DEN-Induced HCC as a Model for Studying Liver Cancer

DEN-induced hepatocellular carcinoma is widely used as an experimental model to study the mechanisms of liver carcinogenesis and evaluate potential therapeutic interventions. The model closely mimics the pathogenesis of human HCC, including the roles of oxidative stress, inflammation, and genetic alterations in tumor development. Studies using DEN-induced HCC have provided valuable insights into the molecular pathways involved in liver cancer and have facilitated the development of novel therapeutic strategies (Onwurah and Eze, 2000).

Hematological Profile in Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is often associated with significant alterations in haematological parameters due to the liver 's central role in metabolism, detoxification, and the production of blood components. These changes can serve as important biomarkers for diagnosing and monitoring the progression of HCC. Below is a detailed discussion of the haematological profile in hepatocellular carcinoma, including the key changes and their clinical significance:

Overview of Haematological Changes in HCC

The liver plays a crucial role in maintaining haematological homeostasis, including the production of clotting factors, the regulation of red blood cell (RBC) metabolism, and the clearance of damaged blood cells. In HCC, liver dysfunction leads to significant alterations in haematological parameters, which can be used to assess the severity of liver damage and the progression of cancer (Adebayo *et al.,* 2011). Common haematological changes in HCC include anaemia, thrombocytopenia, leukocytosis, and alterations in clotting factors.

Anaemia in HCC

Anaemia is a common haematological abnormality in patients with hepatocellular carcinoma. It is characterized by a decrease in red blood cell (RBC) count, haemoglobin (Hb) levels, and haematocrit (Hct). The causes of anaemia in HCC include:

Chronic Inflammation: The inflammatory response associated with HCC leads to the production of cytokines such as interleukin-6 (IL-6) and hepcidin, which inhibit

erythropoiesis (the production of red blood cells) and reduce iron availability (Ordinioha and Brisibe, 2013).

Bone Marrow Suppression: The toxic effects of cancer and its treatment (e.g., chemotherapy) can suppress bone marrow function, leading to reduced RBC production (Patrick-Iwuanyanwu *et al.,* 2011).

Blood Loss: HCC patients may experience gastrointestinal bleeding due to portal hypertension or ruptured varices, further contributing to anaemia (Onwurah and Eze, 2000).

Significance of Haematological Changes in HCC

Haematological changes in HCC serve as important biomarkers for diagnosing and monitoring the progression of liver cancer. These changes can provide valuable information about the severity of liver dysfunction, the presence of complications such as portal hypertension, and the overall prognosis of the disease. For example:

Anaemia and Thrombocytopenia: These abnormalities are associated with advanced HCC and poor prognosis, as they reflect severe liver dysfunction and bone marrow suppression (Adebayo *et al.,* 2011).

Leukocytosis: Elevated WBC counts are often associated with inflammation and infection, which can complicate the management of HCC and worsen patient outcomes (Ordinioha and Brisibe, 2013).

Coagulation Abnormalities: Prolonged PT and reduced fibrinogen levels are indicative of impaired liver function and are associated with increased bleeding risk in HCC patients (Onwurah and Eze, 2000).

METHODOLOGY

Study area

This study will be carried out in the laboratory of biochemistry department Federal University Wukari, Taraba State.

Aparatus/Instruments

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 fresh stembark of *Annona senegalenesis* 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 methanol (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 methanol extract was filtered first through a fresh cotton plug and then through a whatman filters. 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.

Experimental animals

Twenty-five (25) 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

GROUPS	TREATMENT
1	Group one serves as Normal control without treatment
2	Group two serves as Negative control; DEN + No treatment
3	Group 3 serves as Positive Control; DEN + Standard Drug (Silymarin 100 mg/kg bodyweight
4	Animals were induced with DEN and treated with Methanol stembark Extract 200 mg/kg bodyweight
5	Animals were induced with DEN and treated with Methanol stembark Extract 400 mg/kg body weight

Collection of Samples

On completion of the experimental period, animals were anaesthetized with chloroform (2ml/kg). The blood was collected using a syringe and needle with and without EDTA as anticoagulant.

Haematological analysis

The determinations of haematological parameters were carried out using automated haematology analyser (Agile S30 haematology autoanalyzer V2.0, China). Using whole blood, the total red blood cell (RBC) count, haemoglobin (HB) concentration, packed cell volume (PCV), white blood cell (WBC) count and platelet count were determined.

Statistical analysis

In vitro and other parametric assays were performed in triplicate and results are shown as mean ± SD. Antioxidant potential of different assays was determined as IC_{50}. Statistical significance was determined among various treatments with one way ANOVA test. A statistical significance of $P < 0.05$ was considered to be significant.

RESULTS

Effect of methanol stembark extract of Annona senegalensis on haematological indices of N-Nitrosoethylamine induced carcinogenesis

The results presented in Table 1 below revealed the level of White Blood Cell (WBC), Red Blood Cell (RBC), Haemoglobin (HGB), Haematocrit (HCT) and Platelets (PLT). For WBC, a significant ($p< 0.05$) decrease was observed in groups 2 (negative

control), 3 (positive control), and stembark extract-treated groups (groups 4 and 5) compared with group 1 (normal control group). For RBC, there was also a significant ($p< 0.05$) decrease in the level of RBC in group 3 (positive control) and 4 (treated with 200mg/kg stembark extract) compared with group 1 but there was a significant ($p> 0.05$) increase difference in group 2(negative control) and 5 (treated with 400mg/kg stembark extract) when compared to group 1(normal control). HGB showed a significant ($p< 0.05$) decrease in all groups (2,3,4, and 5) compared with group 1 (normal control). PLT showed a significant ($p< 0.05$) decrease in group 3 and group 4, but there was significant ($p> 0.05$) increase in 2 and 5 when compared to group 1 (normal control). HCT were significantly ($p< 0.05$) increased in group 2, 3, and 5 when compared with groups 1 but there was a significant ($p> 0.05$) decrease in group 4 treated with 200mg/kg of stembark extract.

Table 1. Result for haematological analysis

GROUPS	WBC (10^9/L)	RBC (10^{12}/L)	HGB (g/L)	PLT (10^9/L)	HCT (%)
Normal control	5.86 ± 0.15^d	2.36 ± 0.01^c	11.8 ± 0.1^d	16.5 ± 0.1^c	128.66 ± 1.52^b
Negative control	4.7 ± 0.1^b	3.96 ± 0.01^e	9.73 ± 0.2^c	27.7 ± 0.66^e	250.66 ± 26.10^d
Positive control	3.33 ± 0.10^a	1.75 ± 0.01^b	9.53 ± 0.15^c	10.86 ± 0.20^b	246.33 ± 3.05^d
200mg/kg leaf extract	6.76 ± 0.15^e	0.49 ± 0.09^a	7.7 ± 0.1^a	5.43 ± 0.15^a	22.73 ± 0.15^a
400mg/kg leaf extract	5.33 ± 0.15^c	3.63 ± 0.15^d	8.7 ± 0.1^b	22.56 ± 0.15^d	212 ± 11.53^c

Results represent mean ± standard deviation of group results obtained (n=5). Values with different alphabet as superscripts in the same column are statistically significant ($p < 0.05$).

Hemoglobin percentage concentration

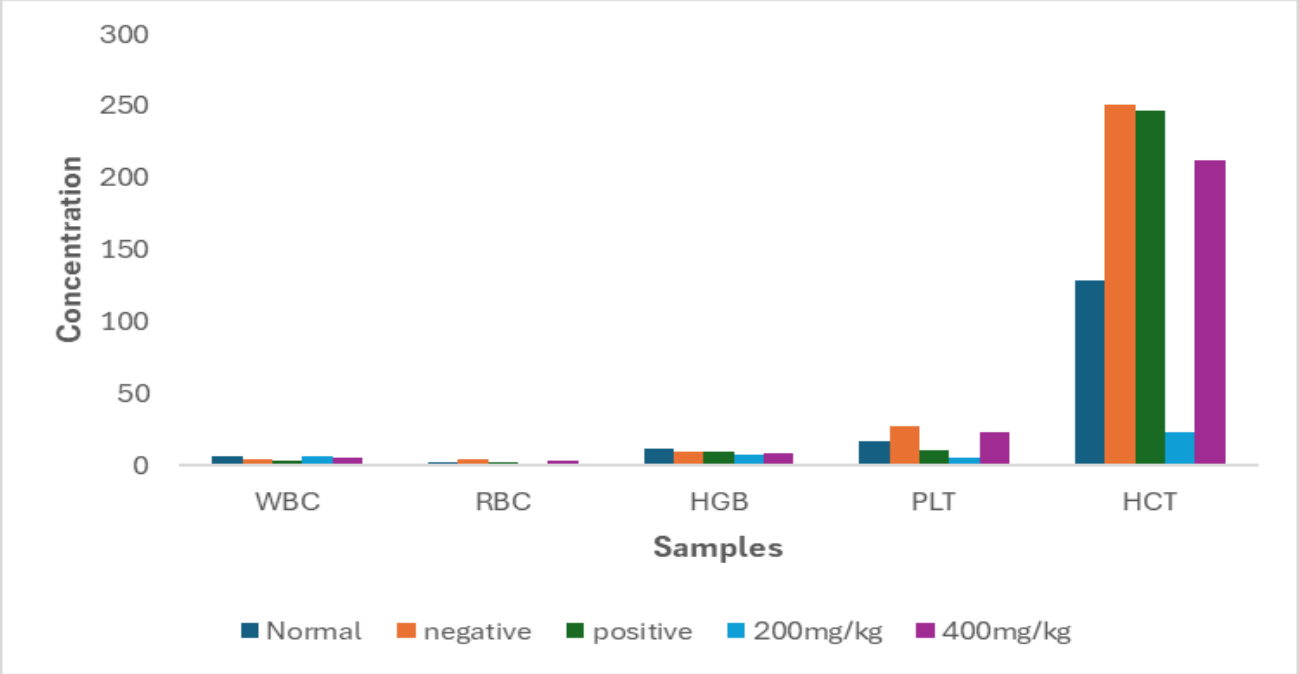

Figure 1 Hemoglobin percentage concentration

DISCUSSION

The results of this study indicate significant alterations in haematological parameters in rats induced with diethylnitrosamine (DEN), a well-established hepatocarcinogen. DEN administration led to marked reductions in red blood cell (RBC) count, haemoglobin (HGB), and platelet (PLT) levels, consistent with previous studies indicating its myelosuppressive effects (Sankar and Villa, 2021). The decrease in RBC and HGB suggests haemolytic anaemia, commonly associated with liver dysfunction. Such haematological disturbances highlight the systemic impact of hepatic carcinogenesis.

Treatment with methanol stem bark extract of *Annona senegalensis* showed variable effects across different doses. The 400 mg/kg treatment significantly improved RBC, HGB, and PLT levels compared to the untreated DEN group, aligning with findings that plant-derived antioxidants can enhance erythropoiesis and counteract oxidative stress-induced haematotoxicity (Vicidomini et al., 2024). This suggests that *Annona senegalensis* may possess haematoprotective properties.

White blood cell (WBC) counts were notably decreased in the DEN-induced groups compared to the normal control. The reduction in WBCs is likely due to immunosuppression induced by carcinogenesis, as reported in previous research (Guo et al., 2021). However, the 200 mg/kg and 400 mg/kg extract treatments significantly restored WBC levels, reinforcing the immunomodulatory role of phytochemicals found in *Annona senegalensis* (Mahmoud et al., 2025). The haematocrit (HCT) levels were significantly decreased in the 200 mg/kg treatment group, suggesting potential haemodilution or ineffective erythropoiesis at this dose.

More also, the 400 mg/kg group exhibited an increase in HCT, further supporting the dose-dependent haematoprotective effects of *Annona senegalensis* (Okoye et al., 2012). Platelet counts followed a similar trend, with higher doses showing potential in mitigating DEN-induced thrombocytopenia, aligning with studies highlighting the pro-thrombopoietic properties of plant bioactives (Amuzat et al., 2020).

The improvement in haematological indices may be attributed to the rich phytochemical composition of *Annona senegalensis*, which includes flavonoids, alkaloids, tannins, and saponins. Flavonoids such as quercetin and kaempferol are known for their antioxidant and anti-inflammatory properties, which counteract oxidative stress-induced haematotoxicity (Alrumaihi et al., 2024). The observed protective effects of *Annona senegalensis* are in line with findings from previous studies on medicinal plants used in hepatic and haematological disorders. Studies on *Silymarin*, a standard hepatoprotective agent, have demonstrated similar protective effects on haematological indices in DEN-induced liver injury models (Mansour et al., 2022). The present study corroborates these findings, suggesting that *Annona senegalensis* may serve as a complementary or alternative therapeutic option.

The ability of *Annona senegalensis* to restore haematological parameters highlights its potential role in managing chemotherapy-induced haematotoxicity. Many conventional chemotherapeutic agents, such as cisplatin and doxorubicin, cause severe bone marrow suppression, leading to anaemia and thrombocytopenia (Mustapha et al., 2022). The haematoprotective effects observed in this study suggest that *Annona senegalensis* could be beneficial in mitigating these side effects. Beyond its haematological effects, *Annona senegalensis* has been shown to exhibit hepatoprotective properties in various studies (Udodeme et al., 2019). Its ability to modulate oxidative stress, reduce inflammation, and

promote liver regeneration makes it a promising candidate for complementary therapy in hepatocellular carcinoma (HCC) management.

The findings from this study indicate that methanol stem bark extract of *Annona senegalensis* significantly ameliorates DEN-induced haematological alterations in Wistar rats. Its ability to restore RBC, HGB, WBC, and PLT levels underscores its potential as a natural haematoprotective agent. These results, supported by existing literature, suggest that *Annona senegalensis* could serve as an adjunct therapy in managing liver cancer-related haematological disorders and chemotherapy-induced myelosuppression. Future research should focus on elucidating its precise mechanisms and assessing its clinical applications.

CONCLUSION

The results of this study show that the methanol extract from the stem bark of *Annona senegalensis* helps to reverse blood abnormalities caused by DEN in Wistar rats. The extract effectively improved levels of red blood cells (RBC), haemoglobin (HGB), white blood cells (WBC), and platelets (PLT), suggesting its potential as a natural remedy for protecting liver health. The observed benefits indicate that *Annona senegalensis* may help reduce liver damage linked to cancer development, likely due to its rich natural compounds and antioxidant properties.

Recommendation

More research should be done to understand how *Annona senegalensis* works to protect the blood and liver, especially in humans.

Safety studies should be conducted to find the right dosage and check for any possible side effects before using *Annona senegalensis* as a treatment.

Clinical trials should be carried out to confirm whether *Annona senegalensis* can be used alongside other treatments to help people with liver cancer manage blood-related issues.

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