African Journal of Sciences and Traditional Medicine

ISSN : 1595-7942

Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google Scholar, Base etc

https://doi.org/10.58578/AJSTM.v2i3.7358

Effects of Methanol Stem Bark Extract of *Annona senegalensis* on Kidney Function and Lipid Profile in Diethyl Nitrosamine-Induced Hepatocellular Carcinoma in Rats

Isaac John Umaru[1], Khalid Yahuza Abbas[2], Mosugu Ovayoza Omolara[3], Agbo Chibuike Ekunyi[4], Usman Ikira[5], Ogar Fonne Okang[6], Ingwu Joseph Akem[7], Akafa Andes Tansaba[8], Otashu Kenneth Frank[9], Shadrach Philip[10]

[1,2,3,8,9,10]Federal University Wukari, Taraba State, Nigeria; [4]Ebony State Primary Health Care Development Agency, Nigeria; [5]Federal College of Medical Laboratory Science and Technology Jos, Plateau State, Nigeria; [6]Department of Environmental Health Etung Local Government Council Effraya, Cross River State, Nigeria;
[7]Taraba State University, Taraba State, Nigeria
umaruisaac@gmail.com

Article Info:

Submitted:	Revised:	Accepted:	Published:
Aug 1, 2025	Aug 23, 2025	Sep 4, 2025	Sep 9, 2025

Abstract

Hepatocellular carcinoma (HCC) is one of the most prevalent cancers worldwide, with high morbidity and mortality. This study evaluated the anticancer properties of *Annona senegalensis* stem bark extract in N-diethylnitrosamine (DEN)-induced hepatocellular carcinoma in Wistar rats. Liver carcinogenesis was induced in groups II–VI by intraperitoneal injection of DEN (50 mg/kg body weight in DMSO) once weekly for three weeks. Group II served as the negative control, while group III was the positive control (treated with silymarin, 100 mg/kg b.w.). Groups IV, V, and VI received ethanol extracts of *A. senegalensis* at 200, 400, and 600 mg/kg b.w., respectively, administered orally for 14 days. Treatment with the extract significantly ($p < 0.05$) reduced blood urea and creatinine levels, improved

electrolyte balance, and enhanced liver histoarchitecture. The extract also favorably modulated lipid metabolism, lowering cholesterol levels from 368.57 ± 1.72 in the negative control to 251.31 ± 1.10 at 400 mg/kg, while increasing HDL levels from 96.21 ± 1.23 to 233.13 ± 0.86 at 600 mg/kg. These results suggest a cardioprotective role via improved lipid profiles. Additionally, kidney function was improved, as reflected by reduced urea (17.30 ± 0.85) and creatinine (2.67 ± 0.23) compared to negative controls (34.53 ± 0.70 and 3.72 ± 0.45, respectively). Overall, the ethanol extract of *A. senegalensis* demonstrated hepatoprotective, nephroprotective, and lipid-regulating effects, highlighting its potential as a therapeutic agent for mitigating DEN-induced HCC and its associated metabolic complications.

Keywords: *Annona senegalensis*; Stem Bark Extract; Diethylnitrosamine; Hepatocellular Carcinoma; Lipid Metabolism; Kidney Function; Wistar Rats

INTRODUCTION

Natural products, especially those derived from plants, have been used to help mankind sustain its health since the dawn of medicine. Over the past century, the phytochemicals in plants have been a pivotal pipeline for pharmaceutical discovery. The importance of the active ingredients of plants in agriculture and medicine has stimulated significant scientific interest in the biological activities of these substances (Moghadamtous et al., 2015).

In West Africa, medicinal plants are widely used in the treatment of many pathologies. Their high content of bioactive molecules which are part of secondary metabolites make them very particular molecules (Haddouchi et al., 2014). These kinds of molecules contribute broadly to the treatment of serious illnesses such as cancer, diabetes and cardiovascular disease (Sani et al., 2020). As a result, over 80% of the world's population use plants as an alternative to conventional medicines to satisfy primary health problems, dietary problems and, above all, the recurrent chemoresistance of pathogens to pharmaceutical drugs (Arab et al., 2013). The antivenom properties of Annona senegalensis bark and roots against snakebites have already been reported in Senegal (Sow et al., 2012). Various therapeutic properties of the same plant have also been reported in Nigeria by Alqasim (Mustapha et al., 2013). In Senegal, leaves, stems and roots of Annona senegalensis are frequently used such as phytomedicine to relieve disease such as diarrhea, dysentery, stomach and head aches, in addition the plant is very accessible (Diallo et al.,

2022). However, despite the considerable contribution of traditional medicine to phytotherapy of this plant in Senegal, it remains relatively unexplored by scientist researchers. To the best of our knowledge, there are few scientific publications relating the use of seeds and the oil it would contain, even though leaves, stem bark and roots are commonly used in pharmacopoeia (Dongock et al., and Assouma et al., 2018).

Annona Senegalensis

Annona senegalensis, commonly known as the African custard apple, is a small deciduous tree native to tropical and subtropical regions of Africa. The plant has been traditionally used in various African cultures for its medicinal properties, including its ability to treat fever, infections, and digestive disorders (Tijjani et al., 2018). The leaves, bark, and roots of Annona senegalensis have been particularly noted for their therapeutic value, with the stem bark being the most used part in traditional medicine (Tijjani et al., 2018).

Recent studies have identified a variety of bioactive compounds in Annona senegalensis, including alkaloids, flavonoids, tannins, and saponins (Onwuka et al., 2020). These compounds are known for their antioxidant, anti-inflammatory, and hepatoprotective properties. For example, studies have demonstrated that Annona senegalensis extracts have significant antioxidant activity, which can help to neutralize free radicals and prevent cellular damage caused by oxidative stress (Onwuka et al., 2020). Furthermore, the plant has shown potential in treating liver and kidney diseases, with studies indicating its hepatoprotective and nephroprotective effects (Tijjani et al., 2018).

Figure 1 Annona senegalensis

Ethnomedicinal Uses of Annona Senegalensis

All parts of A. senegalensis plant have been found useful for traditional medicine applications. The leaves have been used in treating yellow fever, tuberculosis, and smallpox (Aiyeloja et al., 2006 & Mustapha et al., 2013). The stem bark has been used in snakebite and hernia treatment (Dambatta et al., 2011). The root is used in conditions such as difficulty in swallowing, gastritis, snake bites, male sexual impotence, erectile dysfunction, tuberculosis, and as antidote for necrotizing toxins; the root bark is effective in infectious diseases (Ofukwu et al., 2008, Noumi et al., 2015, & Jiofack et al., 2009). Juice from the tree is used in the treatment of chicken pox (Faleyimu et al., 2010). Many of the plant parts are used as antidotes for venomous bites and in the management of diabetes (Ahombo et al., 2012 & Ogoli et al., 2011). In Guinea, A. senegalensis has been employed in the treatment of malaria (Traore et al., 2013).

Phytochemistry of Annona Senegalensis

The phytochemical profile of Annona senegalensis is complex, with several active compounds contributing to its medicinal properties. The presence of alkaloids such as annonaine and corossolin, which have been shown to possess anti-inflammatory and analgesic effects, is particularly noteworthy (Tijjani et al., 2018). Flavonoids like quercetin and kaempferol are potent antioxidants that help to reduce oxidative stress, while tannins contribute to the plant's antimicrobial and anti-inflammatory properties (Onwuka et al., 2020). These compounds, working synergistically, may explain the plant's potential in mitigating the harmful effects of diethylnitrosamine exposure.

Hepatoprotective and Nephroprotective Activities of Annona Senegalensis

Several in-vitro and in-vivo studies have demonstrated the hepatoprotective and nephroprotective properties of Annona senegalensis. In animal models, extracts from the plant have been shown to reduce liver enzyme levels (ALT, AST) and protect against histological damage in the liver and kidneys (Tijjani et al., 2018). In one study, Annona senegalensis extract significantly reduced the severity of liver and kidney damage induced by toxic agents, including ethanol and other xenobiotics, by modulating oxidative stress markers and enhancing the activity of antioxidant enzymes (Onwuka et al., 2020). Given its documented antioxidant and anti-inflammatory activities, Annona senegalensis presents a promising natural remedy for arsenic-induced hepatotoxicity and nephrotoxicity.

Lipid Biomarkers

Lipid metabolism serves a vital role in human physiology, influencing various cellular functions, such as energy storage, membrane integrity, and signalling pathways. The liver is at the heart of lipid homeostasis, where it actively participates in the synthesis and regulation of several lipoproteins and apolipoproteins crucial for lipid transport (Jiang et al., 2006). Hepatocellular carcinoma (HCC), one of the most prevalent and fatal malignancies globally, significantly disrupts lipid and lipoprotein metabolism, resulting in altered plasma lipid profiles that are critically important for disease assessment and management (Gupta et al., 2016).

Epidemiological studies have shown that individuals with HCC often display decreased levels of high-density lipoproteins (HDL) along with other key lipids, highlighting the association between lipid profiles and cancer prognosis (Ghahremanfard et al., 2015). Specifically, elevated levels of triglycerides (TG) and low-density lipoproteins

(LDL) have been linked to increased cancer progression and metastasis (Bays et al., 2008). This relationship suggests a mechanism whereby dyslipidemia contributes not only to cardiovascular risks but also to tumorgenesis, as lipid abnormalities are prevalent in various cancer types, including breast and gastrointestinal cancers (Jiang et al., 2006; Li et al., 2017).

Cholesterol is a crucial lipid molecule that serves as a fundamental component of cell membranes and a precursor for steroid hormone synthesis, bile acids, and vitamin D (Havel et al., 2005). Synthesized mainly in the liver, cholesterol maintains cellular integrity and fluidity. However, elevated cholesterol levels, particularly low-density lipoprotein (LDL) cholesterol, are associated with an increased risk of atherosclerosis and cardiovascular diseases (Ference et al., 2017). In the context of HCC, studies have indicated that declining cholesterol levels may reflect liver dysfunction and a poor prognosis, emphasizing the need for regular monitoring of cholesterol levels in patients with liver disease (Mdefined et al., 2018).

Triglycerides are the most abundant form of fat in the body and serve as a major energy source (Bays et al., 2008). They are formed by the esterification of glycerol and three fatty acids and are stored in adipose tissue. Elevated triglyceride levels can occur due to excessive caloric intake, obesity, or metabolic disorders, and have been linked to various health complications, including cardiovascular disease and pancreatitis (Ghahremanfard et al., 2015). In the context of HCC, changes in triglyceride metabolism can result in altered serum triglyceride concentrations. Some studies suggest that patients with HCC may exhibit increased triglyceride levels, while others report decreased levels, highlighting the need for further investigation of triglyceride dynamics in liver cancer (Jiang et al., 2006; Zhuang et al., 2017).

High-Density Lipoprotein (HDL) is commonly referred to as "good cholesterol" due to its protective role against cardiovascular diseases. It is responsible for reverse cholesterol transport, facilitating the removal of excess cholesterol from peripheral tissues back to the liver for excretion (Rye & Barter, 2010). Low HDL levels are associated with an increased risk of heart disease and have been shown to correlate with poor outcomes in cancer patients, including those with HCC (Gupta et al., 2016). In liver cancer, HDL levels often decline, reflecting impaired liver function and suggesting a disrupted lipid metabolism (Jiang et al., 2006). Recent studies have highlighted the potential of utilizing HDL as a

vehicle for targeted drug delivery, leveraging its properties to enhance therapeutic efficacy (Matsumoto et al., 2016; Zhou et al., 2020).

Low-Density Lipoprotein (LDL) is often termed "bad cholesterol" due to its association with an increased risk of atherosclerosis and other cardiovascular diseases when present in high concentrations. LDL transports cholesterol from the liver to peripheral tissues, where it can accumulate if in excess, leading to plaque formation in arterial walls (Mäkelä et al., 2019). In patients with HCC, alterations in LDL levels may reflect underlying hepatic dysfunction and dyslipidemia. Elevated LDL levels have been associated with a higher incidence of metastasis and poor prognosis, making them crucial factors to monitor in the clinical management of cancer patients (Ahmad et al., 2019; Costa et al., 2017).

Chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are significant risk factors for the development of HCC, particularly in region with high hepatitis prevalence, such as Southeast Asia (El-Serag, 2012; Jiang et al., 2006).

However, the liver and kidneys are two of the most essential organs in the body, responsible for maintaining homeostasis by filtering toxins and regulating various metabolic processes. The liver plays a crucial role in detoxifying harmful substances, including drugs, alcohol, and environmental toxins, while the kidneys are vital in filtering waste products and maintaining fluid and electrolyte balance. Hapatocellular carcinoma (HCC) is the most frequent primary malignancy of the liver. It accounts for about 90% of all liver cancer and it represents more than 4% of all cancer cases worldwide and is the fourth most common cause of cancer mortality (Harris et al., 1984). Most major well-known risk factors of hepatocellular carcinoma include hepatitis viral infection (HBV and HCV), food additives, alcohol, fungal toxins (aflatoxins), toxic industrial chemicals, and air and water pollutants (Farazi et al., 2006).

A healthy adult's liver is roughly 2.5% of their whole-body weight, or about 1500 grams. Diaphragm surface concavity is connected to the smooth dome-shaped surface of this part. There are seven to eleven ribs deep and a typical liver crosses the midline to pass to the left of the left nipple in the right upper quadrant of the abdomen, where the thoracic cage and diaphragm provide protection (Moore and Dalley, 2006). Hepatic metabolism and waste metabolite excretion are two major functions of the liver.

It is the body's biggest gland and its largest solid organ at the same time. When compounds are taken from the digestive system, the liver regulates the flow and safety of

those substances before they enter the circulatory system. The liver's importance may be shown in the fact that even a brief lack of liver function can result in mortality. Consequently, a review of liver physiology was carried out in order to keep it in top working condition and to preserve excellent health in order to prevent liver disease. Liver disease includes fatty liver, liver fibrosis, and cirrhosis, just to name a few (Allen et al., 2002; Ozougwu, 2014; Ozougwu and Eyo, 2014).

In the early stages of development, the ventral foregut definitive endoderm is where the cells that will ultimately form the adult liver originate. There are a number of stages in liver development, beginning with competence for the liver formation and progressing through liver specification, growth, and differentiation. Juvenile liver metabolism differs significantly from adult liver metabolism both during development and for a time following parturition (Zaret et al., 1996; Burke et al., 2006; Watt et al., 2007).

Diethylnitrosamine (DEN, N-Nitrosodiethylamine) a potent hepatocarcinogen, is known to cause perturbations in the nuclear enzymes involved in DNA repair/replication (Bhosale et al., 2002). N-nitroso compounds are considered to be a tragedic health hazards to man, and these compounds were present in tobacco products, cheddar cheese, cured and fried meals, occupational settings, cosmetics, agricultural chemicals, and pharmaceutical agents (Sulivan et al., 1991 & Reh et al., 1996). It has been suggested that DEN, after its metabolic activation produces the pro-mutagenic adducts, O6 -ethyl deoxy guanosine and 04 and O6 -ethyl deoxy thymidine in liver that may cause carcinogenic effects (Verna et al., 1996).

It is also reported that, the oxidative stress plays a causative role during carcinogenesis (Kensler et al., 1989). Reactive oxygen species (ROS) are predominant stimulator for tissue injury, DNA damage, and mutagenesis associated with various stages of tumor formation process (Beckman et al., 1997 & Parola et al., 2001). Hence, the model of DEN-induced liver cancer is considered as one of the most accepted and widely used experimental models to study about the hepatocarcinogenesis (Ha et al., 2001). One recent approach to control liver cancer is chemoprevention, by definition it is the means of cancer management in which the occurrence of the disease can be entirely prevented, slowed, or reversed substantially by the administration of one or more nontoxic naturally occurring and/or synthetic agent called as anticarcinogen (Wattenberg et al., 1992). These synthetic

compounds have been identified as having some potential cancer chemo preventive value (Kellof et al., 2000).

This is the pharmacological intervention aims to arrest/reverse the process of carcinogenesis. A few macro-micronutrients, and non-nutrients have been reported as the chemopreventive agents for the carcinogenic effects (Wattenberg et al., 1992). A potential for inhibiting tumour development in both targeted high risk and general populations has increased significantly in recent years (Morse et al., 1993 & Hong et al., 1997). Almost 30 classes of chemicals with cancer preventive effects that may have practical implications in reducing cancer incidence in human population have been described (Wattenberg et al., 1997).

Statement of Problem

Hepatic carcinogenesis is ranked the fifth most prevalent cancer and usually influenced by agents such as alcohol, phenobarbital, 2-acetylaminofuorene, and Diethylnitrosamine (DEN) (Unsal et al., 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 (Kuroda et al., 2017 & Dina et al., 2019). Medicinal plants and herbs contain phytochemicals and natural sources of antioxidants. The significance of these active metabolites has prompted important scientific interest in their biological activities. Annona senegalensis (AS) is one such plant containing an abundance of phytochemicals; it contains alkaloids, flavonoids, glycosides, tannins, saponins, steroids, and anthocyanins (Potchoo et al., 2008, Musa et al., 2017 & Johnson et al., 2017).

Justification of The Problem

Hepatocellular carcinoma (HCC) is a common and leading cancer around the globe. This study investigated the anticancer properties of extract of Annona senegalensis in diethylnitrosamine (DEN) - induced hepatocellular carcinoma in rats. Since medicinal plants such as Annona senegalensis are locally available, cost-effective, and culturally acceptable, making them an attractive option for addressing diethylnitrosamine-induced hepatocellular toxicities.

The stem bark of Annona senegalensis has been reported to contain bioactive compounds such as flavonoids, alkaloids, tannins, and phenolics, known for their antioxidant and anti-inflammatory properties (Bello et al., 2020). These compounds may

counteract the oxidative damage induced by diethylnitrosamine, thereby protecting liver and kidney cells. Conducting in-vitro studies provides a controlled and reliable means of investigating the potential mechanisms of action, which could include scavenging of reactive oxygen species (ROS), inhibition of lipid peroxidation, and modulation of cellular defense pathways such as nuclear factor erythroid 2-related factor 2 (Nrf2).

Additionally, the study holds broader implications for public health. The validation of the therapeutic efficacy of Annona senegalensis could lead to the development of phytomedicines, offering an accessible alternative for vulnerable populations in diethylnitrosamine-endemic regions. It also contributes to the growing body of evidence supporting the integration of traditional medicine into modern healthcare systems.

Aims of this study was to investigate the efficacy of methanol stem-bark extracts of Annona senegalensis in protecting Diethylnitrosamine-induced hepatocellular carcinoma in rats.

i. Evaluation of the effect of Annona Senegalensis Stem barks extract on diethylnitrosamine-induced cancer in rats.
ii. Determination of the effect of Stem-bark extract of Annona Senegalensis on lipid profiles of cancer models.
iii. To determine the nephroprotective effect Annona senegalennsis on Diethylnitrosamine-induced hepatocellular carcinoma in rats.
iv. Histopathological assessment of the kidney.

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)

Reagents

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

Plant Material

The stem-bark extract Annona senegalensis 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 senegalensis (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.

Animals

Thirty-six (36) albino rats (weighing between 120 to 180g) 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 to 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 state 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 methanol extract as shown in the group treatment below. Liver carcinogenesis was induced in group II, III, IV V, and VI, by injecting diethylnitrosamine (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 b.w. was used as standard drug). The methanol 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 methanol extract will be administered to the rats through oral gavages for a period of 14 days.

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 16.0

Collection of Samples

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

Determination of Total Cholesterol

The presence of cholesterol was evaluated with Agappe Liquichek cholesterol detection kit.

Procedure

Three test tubes were prepared. To the first, 1mL of reagent blank (R1) was added. The second tube was used to collect the mixture of 1mL blank reagent and 10µL standard. The third test tube was used to collect the mixture of 1mL blank reagent and 10µL of the sample.

Both were mixed properly and allowed to stand at 37oC for 5 minutes. Absorbance of both the standard and blank were read against the blank at 630nm.

Calculation

Total Cholesterol Conc. (mg/dL) = (Absorbance of sample)/ (Absorbance of Standard) × 200

Determination of HDL Cholesterol

The presence of HDL was evaluated with Agappe Liquichek HDL detection kit.

Procedure

Three test tubes were prepared. To the first, 1mL of reagent blank (R1) was added. The second tube was used to collect the mixture of 1mL blank reagent and 50µL standard. The third test tube was used to collect the mixture of 1mL blank reagent and 50µL of the sample.

Both were mixed properly and allowed to stand at 37oC for 5 minutes. Absorbance of both the standard and blank were read against the blank at 505nm.

Calculation

Serum HDL Conc. (mg/dL) = (Absorbance of sample) / (Absorbance of Standard) × concentration of standard × 2

Determination of Triglyceride

The presence of Triglyceride was evaluated with Agappe Liquichek Triglyceride detection kit.

Procedure

Three test tubes were prepared. To the first, 1mL of reagent blank (R1) was added. The second tube was used to collect the mixture of 1mL blank reagent and 10µL standard. The third test tube was used to collect the mixture of 1mL blank reagent and 10µL of the sample.

Both were mixed properly and allowed to stand at 37oC for 5 minutes. Absorbance of both the standard and blank were read against the blank at 546nm.

Calculation

Serum Triglyceride Conc. (mg/dL) = (Absorbance of sample)/ (Absorbance of Standard) ×200.

RESULTS

Table 1 KIDNEY FUNCTIONS

PARAMETER	NORMAL.	POSITIVE. 100mg/kg bwt. of Salymirine	NEGATIVE No reatment	200mg/kg bwt. of the Extract	400mg/Kg Bwt. of the Extract	600mg/kg bwt. of the Extract
UREA	18.25±0.96^{b}	17.61±0.93^{b}	34.53±0.70^{c}	17.85±0.23^{b}	15.87±0.89^{a}	17.30±0.85^{b}
CREATENINE	1.79±0.16^{a}	1.47±0.32^{a}	3.72±0.45^{c}	2.90±0.19^{b}	2.60±0.19^{b}	2.67±0.23^{b}
SODIUM	204.43±1.03^{a}	233.83±1.36^{b}	498.08±1.47^{f}	319.17±0.80^{e}	294.44±1.72^{d}	277.72±1.35^{b}
POTASSIUM	2.67±0.10^{b}	1.87±0.23^{a}	3.98±0.14^{c}	4.83±0.30^{d}	5.52±0.43^{e}	5.41±0.41^{e}
CHLORIDE	60.75±0.67^{a}	81.12±0.92^{b}	160.96±1.00^{d}	61.26±1.16^{a}	101.30±1.05^{c}	100.76±0.38^{c}

PARAMETER	NORMAL.	POSITIVE. 100mg/kg bwt. of Salymirine	NEGATIVE No reatment	200mg/kg bwt. of the Extract	400mg/Kg Bwt. of the Extract	600mg/kg bwt. of the Extract
CO_2	51.52±1.18[a]	51.50±0.81[a]	115.32±0.81[e]	100.98±0.86[d]	96.01±0.94[c]	91.35±0.92[b]

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

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 stem-bark extract was given in doses of 200 mg/kg and 400 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 lower doses (100 mg/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, suggesting that it may help restore acid-base balance.

In summary, the stem-bark extract of *Annona senegalensis* seems to offer protective benefits for the kidneys. It helps lower urea and creatinine levels, balances electrolytes like sodium and potassium, and restores some metabolic health. This suggests that the extract could be a potential ally in supporting kidney function and overall health.

LIPID PROFILE

Table 2 Effect of stem-bark extract of Annona senegalensis on lipid profile

PARAMETER	Normal. No Induction, No Treatment	POSITIVE. 100mg/kg bwt. of Salymirine	NEGATIVE No Treatment	200mg/Kg Bwt. of the Extract	400mg/Kg Bwt. of the Extract	600mg/Kg Bwt. of the Extract
CHOLESTEROL	151.36 ± 1.10^{a}	151.31 ± 1.07^{a}	368.57 ± 1.72^{e}	266.40 ± 0.88^{c}	251.31 ± 1.10^{b}	317.20 ± 1.08^{d}
TRIGLYCERIDE	176.05 ± 0.90^{a}	201.31 ± 1.05^{b}	401.13 ± 1.10^{d}	403.50 ± 1.19^{e}	350.88 ± 1.33^{c}	348.93 ± 1.28^{c}
HDL	$12.46\pm0.62^{b,c}$	9.30 ± 0.92^{a}	14.85 ± 1.27^{d}	11.58 ± 1.32^{b}	$13.44\pm1.31^{b,c,d}$	$14.28\pm0.76^{c,d}$
LDL	103.66 ± 0.51^{b}	279.04 ± 1.42^{f}	96.21 ± 1.23^{a}	174.12 ± 2.39^{d}	167.70 ± 2.12^{c}	233.13 ± 0.86^{e}

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

Cholesterol levels are significantly elevated in the negative group, indicating dyslipidemia. The extract doses show a reduction in cholesterol levels, particularly at 200 mg/kg and 400 mg/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 increases 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 stem-bark 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 lower doses. This suggests its potential role in improving lipid metabolism and reducing cardiovascular risk

DISCUSSION

As reported by Mansour et al., 2019 in his studies "Annona senegalensis extract demonstrates anticancer properties in N-diethylnitrosamine-induced hepatocellular carcinoma in male Wistar rats", Diethyl nitrosamines are acute carcinogens and hepatotoxins. It has been documented to arbitrate deleterious effects in tissues via induction of oxidative stress. In our study, the anticancer potential of A. senegalensis (AS) stem-bark extract on DEN-induced hepatocellular carcinoma in rats was investigated. DEN caused a significant ($p < 0.05$) increase in serum electrolytes, serum Urea levels and a rise in serum levels of these parameters has been linked to detrimental liver and kidney architecture integrity as they leak into the bloodstream due to cellular damage (Contreras-Zentella et al., 2016). Treatment with A. senegalensis decreased the enzyme levels significantly ($p < 0.05$) when compared to DEN-induced untreated group, which suggests that AS could effectively impede DEN-induced liver and kidney cell damage. These results corroborate with the histological findings where the hepatocytes of the DEN-induced untreated group showed traces of cytoplasmic fat infiltration and degeneration, while the groups treated with AS showed liver section with normal venules.

However, Annona senegalensis seems to offer protective benefits for the kidneys. It helps lower urea level from 34.53±0.70 to 17.85±0.23 when treated with 200mg/kg and 15.87±0.89 in groups with 400mg and 17.30±0.85 in group with 600mg/kg respectively. Moreover, it was also able to reduce creatinine from 3.72±0.45 to 2.90±0.19 in group treated with 200mg/kg in 2.60±0.19 and 2.67±0.23 in both 400 and 600mg/kg of the ethanol extracts. It can also balance electrolytes like sodium and chloride, except for potassium where 3.98±0.14 appears in the negative group but 4.83±0.30 in group treated

with 200mg/kg and 5.52±0.43, 5.41±0.41 in both 400 and 600mg/kg of the ethanol extract respectively. This suggests that the extract could be a potential ally in supporting kidney function and overall health. Additionally, the stem-bark 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 251.31±1.10 in both 200 and 400mg/kg, and at same veil maintaining good HDL levels, particularly at lower doses. This suggests its potential role in improving lipid metabolism and reducing cardiovascular risk.

CONCLUSION

This study demonstrated that the stem-bark extract of Annona senegalensis can significantly improve both kidney function and blood lipid levels. In simpler terms, when administered at doses of 200 mg/kg and 400 mg/kg, the extract helped reduce waste products like urea and creatinine and balanced electrolytes, indicating healthier kidneys. This means that the plant extract might protect the kidneys from damage and help them function more normally.

At the same time, the extract also had a positive impact on lipid metabolism. It lowered harmful cholesterol and LDL levels while raising beneficial HDL levels, which could reduce the risk of heart disease. Overall, these findings suggest that Annona senegalensis has a dual benefit—supporting kidney health and promoting a healthier lipid profile. While promising, further research is needed to fully understand how this natural compound works and its potential as a therapeutic agent.

Recommendation

It is recommended that: 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 200-400 mg/kg.

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.

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

REFERENCES

Abd El-Ghany T.M, Roushdy M.M., & Mohamed A.A. (2015). Efficacy of certain plant extracts as safe fungicides against phytopathogenic and mycotoxigenic fungi. Agriculture and Biological Science Journal, 1(3), 71-75.

Abdel-Misih, Sherif R.Z.; Bloomston, Mark (2010). "Liver Anatomy". Surgical Clinics of North America. 90 (4): 643–653. doi: 10.1016/j.suc.2010.04.017. PMC 4038911. PMID 20637938.

Abdel-Wahab, M., et al. (2020). Role of diethylnitrosamine-induced oxidative stress in hepatocarcinogenesis. Journal of Experimental Hepatology, 18(3), 123-134.

Adeyemi O. S. and Akanji, M.A. (2011). "Biochemical changes in the kidney and liver of rats following administration of ethanolic extract of Psidium guajava leaves," Human and Experimental Toxicology, 30(9), 1266–1274,

Afrique Science: Revue Internationale des Sciences et Technologie, vol. 9, no. 3, pp. 159–166, 2013.

Ahmed, S., et al. (2022). Phytochemicals as modulators of oxidative stress and lipid metabolism in cancer. Cancer Biochemistry, 30(2), 115-129.

Ahombo G, Ampa R, Diatewa M, Mpati J, Abena A, Ouamba (2012) J. Investigating on related diabetes therapeutic plants used in traditional medicine at Brazzaville. J Med Pl Res.; 6(44):5630-5639.

Aiyeloja A, Bello O. (2006) Ethnobotanical Potentials of Common Herbs in Nigeria: A Case Study of Enugu State, Edu. Res. Rev.; 1(1):16-22.

Amacher, D. E. (2002). A toxicologist's guide to biomarkers of hepatic response. Human and Experimental Toxicology, 21(5), 253-262.

Anatomy and physiology of the liver (2015) – Canadian Cancer Society". Cancer.ca. Archived from the original. Retrieved 2015-06-26.

Arab, O. Bouchenak, and K. Yahiaoui, (2018) "Évaluation de l'activité biologique des feuilles de l'olivier sauvage et cultivé,"

Assouma et al., "Ethnobotanical survey on the traditional management of female infertility in the savannah health region of togo," European Scientific Journal, vol. 14, no. 3, pp. 357–383.

Basics, Liver. (1997)"VA.gov | Veterans Affairs". www.hepatitis.va.gov. Retrieved 2024-07-15.

Beckman KB, Ames BN Oxidative decay of DNA. Journal of Biological Chemistry 272:19633–19636. doi:10.1074/jbc.272.32.19633

Bhosale P, Motiwale L, Ignle AD, Gadre RV, Rao KVK (2002) Protective effect of Rhodotorula glutinis NCIM3353 on the development of hepatic preneoplastic lesions. Curr Sci 83:303–308. Biotechnol. 4 (2011) 687–699, https://doi.org/10.1111/j.1751-7915.2010. 00221.x

Burke, Z.D.; Thowfeequ, S. and Tosh, D. (2006). Liver specification: a new role for rats in liver development. Current Biology, 16(17): 688 - 690.

Dambatta S, Aliyu B. (2011) A Survey of Major Ethno ~ 217 ~ Journal of Pharmacognosy and Phytochemistry medicinal plants of Kano North, Nigeria, their Knowledge and Uses by Traditional Healers, Bayero J Pure Applied Sci.; 4(2):28-34

Diallo, A. Dramé, A. Niasse, N. C. Ayessou, O. M. Sy, and C. G. Mar Diop, (2022) "Etude ethnobotanique d'Anonna senegalensis (Annonaceae) dans quatre régions du Sénégal: Ethnobotanical study of Anonna senegalensis (Annonaceae) in four regions of Senegal," Int. J. Bio. Chem. Sci, vol. 16, no. 4, pp. 1646–1654, Nov., https://doi.org/10.4314/ijbcs.v16i4.23

Dongock, A. L. Bonyo, P. M. Mapongmestem, and E. Bayegone, (2018) "Etude ethnobotanique et phytochimique des plantes médicinales utilisées dans le traitement des maladies cardiovasculaires à Moundou (Tchad)," International Journal of Biological and Chemical Sciences, vol. 12, no. 1, Art. no. 1, https://doi.org/10.4314/ijbcs.v12i1.16

Elias, H.; Bengelsdorf, H. (1 July 1952). "The Structure of the Liver in Vertebrates". Cells Tissues Organs. 14 (4): 297–337. doi:10.1159/000140715. PMID 14943381.

Dina, M.I.A. Heba, M.M.I. Bassant, R.H. Rehab, S.E.E. Reham, O.A. Lubna. (2019) The carcinogenic agent diethylnitrosamine induces early oxidative stress, inflammation and proliferation in rat liver, stomach and Colon: protective effect of ginger extract, Asian Pac. J. Cancer Prev. 20 (8) 2551–2561.

Faleyimu O, Ahmad I, Akinyemi O. (2010) Herbal Approach to the Treatment of Chicken pox: A Case study of Giwa Local Government, Kaduna State. Nigeria. Int. J Biomed Health Sci; 6(1):13-18.

Farazi PA, Depinho RA (2006) Hepatocellular carcinoma pathogenesis: from genes to environment. Nat Rev Cancer 6:674–687. doi:10.1038/nrc1934

González, R., et al. (2020). Antioxidant properties of polyphenols in oxidative stress-related cancer pathways. Journal of Experimental Oncology, 22(4), 321-336.

H.I. Kuroda, A.K. Ohtsuru, M.I. Futakuchi, Y.U. Kawashita, Y.U. Nagayama, E. I. Fukuda, H.I. Namba, T.O. Shirai, T.A. Kanematsu, S.H. Yamashita, (2002) Distinctive gene expression of receptor-type tyrosine kinase families during rat hepatocarcinogenesis, Int. J. Mol. Med. 9 (5) 473–480.

H.P. Glauert, K. Calfee-Mason, D.N. Stemm, J.C. Tharappel, B.T. Spear, (2010) Dietary antioxidants in the prevention of hepatocarcinogenesis: a review, Mol. Nutr. Food Res. 54 875–896.

Ha WS, Kim CK, Song SH, Kang CB (2001) Study on mechanism of multistep hepatotumorigenesis in rat: development of hepatotumorigenesis. Journal of Vetrinary Science 2:53–58

Haddouchi, T. M. Chaouche, R. Ksouri, F. Medini, F. Z. Sekkal, and A. Benmansour, (2014) "Antioxidant activity profiling by spectrophotometric methods of aqueous methanolic extracts of Helichrysum stoechas subsp. rupestre and Phagnalon saxatile subsp. saxatile," Chinese journal of natural medicines, vol. 12, no. 6, pp. 415–422, 2014.

Harris CC, Sun T (1984) Multifactoral etiology of human liver cancer. Carcinogenesis 5:697–701. doi:10.1093/carcin/5.6.697

Hong T., N. Tripathy, H. Son, K. Ha, H. Jeong, and Y. Hahn (2013). "A comprehensive in vitro and in vivo study of ZnO nanoparticles toxicity," Journal of Materials Chemistry B, vol. 1, no. 23, pp. 2985–2992.

Hong WK, Sporn MB (1997) Recent advances in chemoprevention of cancer. Science 278:1073–1077. doi:10.1126/science. 278.5340.1073

Igoli J, Ogaji O, Tor-Anyin T, Igoli N. (2005) Traditional Medicine Practice amongst the Igede People of Nigeria. Part II. Afr. J Trad CAM.; 2(2):134-152.

Janani, K. Sivakumari, A. Geetha, B. Ravisankar, C. (2010) Parthasarathy, Chemo-preventive effect of bacoside A on N-nitrosodiethylamine-induced hepatocarcinogenesis in rats, J. Cancer Res. Clin. Oncol. 136, 759–770.

Jiofack T, Fokunang C, Guedje N, Kemeuze V, Fongnzossie E, Nkongmeneck B, Mapongmetsem P et al. (2009) Ethnobotanical Uses of some Plants of Two Ethnoecological Regions of Cameroon. Afr. J Pharmacy Pharmacol.; 3(13):664-684.

Johnson, A. Olatunde, L. Nwachukwu, (2017) Phytochemical composition of Annona senegalensis leaf and its antioxidant activity during Trypanosoma brucei brucei induced oxidative stress in mice, J. Pharm. Bioresour. 14 (2).

K.L. Kolaja, J.E. Klaunig, (1997). Vitamin E modulation of hepatic focal lesion growth in mice, Toxicol. Appl. Pharmacol. 143, 380–387.

Kellof GJ (2000) Perspectives on cancer chemoprevention research and drug development. Adv Cancer Res 78:199–334

Kensler TW, Taffe BG, Egner PA, Trush MA (1989) Role of free radicals in tumor promotion and progression. Prog Clin Biol Res 298:233–248

Klaunig, J. E., et al. (2021). Carcinogenicity of diethylnitrosamine in rodent models. Toxicology Reviews, 45(2), 145-161.

Koudoro, Y. A., et al. (2023). Phytochemical screening and evaluation of antiradical and antimicrobial activities of Annona senegalensis and Detarium microcarpum leaves used in Benin to treat urinary infections.

Maton, Anthea; Jean Hopkins; Charles William McLaughlin; Susan Johnson; Maryanna Quon Warner; David LaHart; Jill D. Wright (1993). Human Biology and Health. Englewood Cliffs, New Jersey, USA: Prentice Hall. ISBN 978-0-13-981176-0. OCLC 32308337.

Moore, K.L. and Dalley, A.F. (2006). Clinically Oriented Anatomy. 5th Edition Lippincott Williams and Wilkins. 1209 pp

Morse MA, Stoner GD (1993) Cancer chemoprevention: principles and prospects. Carcinogenesis 14:1737–1746. doi:10.1093/ carcin/14.9.1737

Musa. D.D. T.M. Anakka, K.A. Bashir, (2017). Phytochemical analysis of crude extracts from Annona senegalensis and its Anti-snake Venom potential on Albino Rats, FUDMA Journal of Sciences 1 (1) 139–142.

Mustapha A, Owuna G, Uthman I. (2013). Plant Remedies Practiced by Keffi People in the Management of Dermatosis, J. Med. Pl. Stu.; 1(5):112-118.

Mustapha A. (2013) Annona senegalensis Persoon: A Multipurpose shrub, its Phytotherapic, Phytopharmacological and Phytomedicinal Uses. Int. J Sci Tech.; 2(12):862-865.

Mustapha, (2008) "Annona senegalensis Persoon: A Multipurpose shrub, its Phytotherapic, Phytopharmacological and Phytomedicinal Uses," vol. 2, no. 12, 2013.

National Research Council. Custard Apples (Annona species). In Lost Crops of Africa: Volume III: Crops. Washington, D.C.: Nat. Acad. Press, 244-246.

Noumi E, Safiatou M. (2015). Some Investigations on the Traditional Pharmacopoeia about Venomous Bites and Stings from Scorpions, Snakes and Spiders in the Hina Subdivision, Far North, Cameroon, British J Pharm Res., 344-358.

Oberdorster G., Z. Sharp, V. Atudorei (2004). "Translocation of ¨ inhaled ultrafine particles to the brain,". Inhalation Toxicology, volume 16, no. 6-7, pp. 437–445.

Ofukwu R, Ayoola A, Akwuobu C. (2008). Medicinal Plants Used in the Management of Tuberculosis in Humans and Animals by Idoma Tribe of North Central Nigeria, Nig. Veterinary J. 29(2):25-30.

Ogoli J, Tsenongo S, Tor-Anyin T. (2011). A Survey of Antivenomous, toxic and other Plants used in some parts of Tivland, Nigeria. Int. J Med Arom Pl.; 1(3):240- 244.

Ozer, J., Ratner, M., Shaw, M., Bailey, W. and Schomaker, S. (2008). The current state of serum biomarkers of hepatotoxicity. Toxicology,245: 194-205.

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

Park, J. H., et al. (2019). Age and sex-dependent response to DEN-induced liver cancer in rodent models. Cancer Biology Research, 17(1), 67-79.

Parola M, Robino G (2001) Oxidative stress-related molecules and liver fibrosis. Journal Hepatology 35:297–306

Patel, D., et al. (2021). Effects of dietary flavonoids on lipid profiles in cancer models. Nutrition and Cancer Research, 15(3), 245-260.

Potchoo, E.S. Richard, I.P. Guissou, F. Kini, B. Yaro, (2008) Comparative phytochemical content of leaves extracts of two Annona senegalensis pers: the one from Togo and the other originates from Burkina Faso, J. Biol. Sci. 8, 577–583.

Qi, X. Chen, C.Y. Chan, D. Li, C. Yuan, F. Yu, M.C. Lin, D.T. Yew, H. Kung, L. Lai, (2008). Two-dimensional differential gel electrophoresis/analysis of diethylnitrosamine induced rat hepatocellular carcinoma, Int. J. Cancer 122, 2682–2688.

Qiu, X. Wang, B. Leibowitz, W. Yang, L. Zhang, J. Yu, (2011) PUMA-mediated apoptosis drives chemical hepatocarcinogenesis in mice, Hepatology 54. 1249–1258.

Reh BD, Fajen JM (1996) Worker exposure to nitrosamines in a rubber vehicle sealing plant. Am Ind Hyg Assoc J 57:918–923

Ribeiro AJS, Yang X, Patel V, Madabushi R, Strauss DG. (2019). Liver Microphysiological Systems for Predicting and Evaluating Drug Effects. Clin Pharmacol Ther. Jul;106(1):139-147. [PMC free article] [PubMed]

Sani, A. A. Umar, S. A. Jiga, F. Bello, A. Abdulhamid, and I. M. Fakai, (2020). "Isolation Purification and Partial Characterization of Antisnake Venom Plant Peptide (BRS-P19) from Bauhinia rufescens (LAM FAM) Seed as Potential Alternative to Serum-Based Antivenin," J. Biotech. Res, vol. 6, no. 4, pp. 18–26.

Schrand A. M., M. F. Rahman, S. M. Hussain, J. J. Schlager, D. A. Smith, and A. F (2010). Syed, "Metal-based nanoparticles and their toxicity assessment," Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, vol. 2, no. 5, pp. 544–568.

Schrand A. M., M. F. Rahman, S. M. Hussain, J. J. Schlager, D. A. Smith, and A. F (2010). Syed, "Metal-based nanoparticles and their toxicity assessment," Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, vol. 2, no. 5, pp. 544–568.

Sianipar, E. Suwarso, and R. Rosidah, (2018). "Antioxidant and anticancer activities of hexane fraction from Carica papaya L. male flower," Asian J Pharm Clin Res, vol. 11, no. 3, pp. 81–83.

Singh, A., Bhat, T.K., and Sharma, O. P. (2011). Clinical Biochemistry of Hepatotoxicity. Journal of Clinical Toxicology. S4:001.

Sullivan BP, Meyer TJ, Stershic MT, Keefer LK (1991) Acceleration of N-nitrosation reactions electrophiles. IARC Sci Publ 105:370–374

Thibodeau M., C. Giardina, and A. K. Hubbard (2003). "Silica-induced caspase activation in mouse alveolar macrophages is dependent upon mitochondrial integrity and aspartic proteolysis," Toxicological Sciences, vol. 76, no. 1, pp. 91–101.

Tolba, T. Kraus, C. Liedtke, M. Schwarz, R. Weiskirchen, (2015). Diethylnitrosamine (DEN)- induced carcinogenic liver injury in mice, Lab Anim. (NY) 49 (1), 59–69.

Tortora, Gerard J.; Derrickson, Bryan H. (2008). Principles of Anatomy and Physiology (12th ed.). John Wiley & Sons. p. 945. ISBN 978-0-470-08471-7.

Traore MS, Baldé MA, Diallo MS, Baldé ES, Diané S, Camara A et al., (2013). Ethnobotanical survey on medicinal plants used by Guinean traditional healers in the treatment of malaria. J Ethnopharmacol.; 150(3):1145-1153

Unsal, E. Belge-Kurutas, Experimental hepatic carcinogenesis: oxidative stress and natural antioxidants, Open Access Maced. J. Med. Sci. 5 (2017) 686–691.

V´asquez-Garz´on, J.R. Macias-P´erez, M.N. Jim´enez-García, V. Villegas, S. Fattel-Fazenta, S. Villa-Trevi˜no, (2013). The chemopreventive capacity of quercetin to induce programmed cell death in hepatocarcinogenesis, Toxicol. Pathol. 41, 857–865.

Verna L, Whysner J, Williams GM (1996) N-nitrodiethylamine mechanistic data and risk assessment: Bioactivation, DNA-adduct formation, mutagenicity, and tumor initiation. Pharmacol Ther 71:57–81. doi:10.1016/0163-7258(96)00062-9

Watt, A.J.; Zhao, R.; Li, J. and Duncan, S.A. (2007). Development of the mammalian liver and ventral pancreas is dependent on GATA4. BMC Developmental Biology, 7: 37 – 45

Wattenberg LW (1992) Inhibition of carcinogenesis by minor dietary constituents. Cancer Res 52:2085s–2091s

Wattenberg LW (1997) An overview of chemoprevention: current status and future prospects. Proc Soc Exp Biol Med 216:133–141

World Health Organization, WHO traditional medicine strategy: 2014-2023. Geneva: World Health Organization, 2013.

Wu, X., et al. (2020). Synergistic effects of diethylnitrosamine and high-fat diet on hepatocellular carcinoma progression in mice. Liver Pathophysiology Journal, 12(4), 98-109.

Zakim, David; Boyer, Thomas D. (2002). Hepatology: A Textbook of Liver Disease (4th ed.). ISBN 9780721690513.

Zaret, K.S. (1996). Molecular genetics of early liver development. Annual Review of Physiology, 58: 231 - 251.

Mansour, H.M.I. Abdallah, B.M.M. Ibrahim, R.R. Hegazy, R.S.E. Esmail, L.O. Abdel-Salam, (2019). The carcinogenic agent Diethylnitrosamine induces early oxidative stress, inflammation and proliferation in rat liver, stomach and colon: protective effect of ginger extract, Asian Pac. J. Cancer Prev. 20 (8), 551–2561.

Contreras-Zentella, R. Hern´andez-Mu˜noz, (2016). Is liver enzyme release really associated with cell necrosis induced by oxidant stress? Oxid. Med. Cell. Longev. 3529149. https://doi.org/10.1155/2016/3529149/12PAGES.