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Effect of Methanol Leaf Extract of *Annona senegalensis* on Liver Function in Diethylnitrosamine-Induced Hepatocellular Carcinoma in Rats and Its Potential as an Antibacterial Agent

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Abstract

Hepatocellular carcinoma (HCC) is among the most prevalent and deadly cancers globally, with limited treatment options and poor prognosis in advanced stages. This study evaluated the therapeutic potential of methanol leaf extract of *Annona senegalensis* against diethylnitrosamine (DEN)-induced HCC in Wistar rats and investigated its antibacterial properties. Thirty-six male albino rats were allocated into six groups: Group I (normal control) received no treatment; Group II (negative control) was induced with HCC using DEN (50 mg/kg b.w., intraperitoneally) weekly for three weeks without therapy; Group III (positive control) received DEN followed by silymarin (100 mg/kg b.w.); while Groups IV, V, and VI received DEN followed by *A. senegalensis*

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extract at 200, 400, and 600 mg/kg b.w., respectively, administered orally for 14 days. Biochemical analysis showed that the extract significantly ($p < 0.05$) ameliorated DEN-induced hepatotoxicity, with the 400 mg/kg dose most effective in reducing AST (48.71 ± 1.21 IU/L) and ALT (20.74 ± 1.06 IU/L) compared to the negative control (69.52 ± 0.88 IU/L and 54.97 ± 1.54 IU/L, respectively). Hepatic synthetic function was restored, as indicated by increased total protein (6.37 ± 0.13 g/L) and albumin (5.11 ± 0.10 g/L). Histopathological analysis confirmed marked architectural recovery with reduced necrosis and inflammatory infiltration. The extract also demonstrated significant antibacterial activity, exhibiting dose-dependent inhibition zones against Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria, with effects comparable to standard antibiotics. These findings suggest that *A. senegalensis* leaf extract exerts hepatoprotective effects—likely through antioxidant, anti-inflammatory, and antiproliferative mechanisms—while also offering broad-spectrum antimicrobial benefits. The 400 mg/kg dose emerged as the most effective, highlighting its promise as a cost-effective complementary therapy for managing HCC and bacterial infections, particularly in resource-constrained settings. Further research should focus on isolating and characterizing the bioactive compounds and elucidating their mechanisms of action.

Keywords: Leaf Extract; *Annona senegalensis*; Hepatocellular Carcinoma; Diethylnitrosamine; Liver Function; Wistar Rats; Antibacterial Potential

INTRODUCTION

Natural products refer to chemical compounds produced by plants and animals or naturally occurring substances with specific pharmacological effects (Umaru, 2023). Since the earliest days of medicine, plant-derived natural products have played a crucial role in maintaining human health. Over the last century, plant phytochemicals have been a key resource for pharmaceutical advancements. The biological activities of these compounds have garnered significant scientific attention due to their relevance in both medicine and agriculture (Okhale et al., 2016).

One plant rich in phytochemicals is Annona senegalensis Persoon (Annonaceae), commonly known as Wild Custard Apple or Wild Soursop. This species grows as a shrub or small tree, typically between 2–6 meters tall, though it can reach up to 11 meters under optimal conditions. It has a smooth to slightly rough silver-grey or grey-brown bark. Its leaves are arranged alternately, with an oblong, ovate, or elliptical shape, green to bluish-

green in color, with little to no hairs on the upper surface but often bearing brownish hairs underneath. The flowers, measuring up to 3 cm in diameter, appear singly or in clusters of 2–4 on 2 cm-long stalks. The plant produces fleshy, lumpy, egg-shaped fruits, 2.5–5 cm by 2.5–4 cm in size, initially green and turning yellow to orange when ripe (Okhale et al., 2016).

This species thrives in semi-arid to sub-humid regions of Africa, growing along riverbanks, in fallow lands, swamps, forests, and coastal areas. It is typically found as a solitary plant in the understory of savannah woodlands (Okhale et al., 2016). In Nigeria, Annona senegalensis is widespread, particularly in the northern states of Nasarawa, Kaduna, Kano, Plateau, and Niger, as well as in the Federal Capital Territory, Abuja. It is commonly referred to as "Gwándàn dààjìì"in Hausa and "dukuu-hi "in Fulani (Okhale et al., 2016)

Annona senegalensis, also known as "Wild Custard Apple,"is a shrub or small tree that is widely distributed across Africa. In Northern Nigeria, a decoction made from the plant is traditionally used to treat sleeping sickness. Scientific research has demonstrated that extracts from its leaves possess antimicrobial, antimalarial, antitrypanosomal, antioxidant, and free radical scavenging properties (Titilayo et al., 2017).

The plant exhibits various pharmacological activities, including antimalarial, analgesic, anti-inflammatory, antioxidant, and antimicrobial effects. Additionally, its root bark has been reported to have anti-inflammatory and antiulcer properties and was recently analyzed for its anti-enter pooling compounds (Yusuf et al., 2023).

Liver cancer continues to be a significant global health issue, with cases projected to exceed one million by 2025. Hepatocellular carcinoma (HCC) is the most prevalent type, accounting for approximately 90% of liver cancer cases. Chronic infections with hepatitis B and C viruses are the primary risk factors for HCC development. However, in Western countries, non-alcoholic steatohepatitis (NASH), often linked to metabolic syndrome or diabetes mellitus, is increasingly recognized as a contributing factor (Llovet et al., 2021). Diethylnitrosamine (DEN), a potent liver carcinogen, is commonly used in research to induce HCC in animal models, providing a crucial framework for studying liver cancer and evaluating potential treatment strategies.

Statement of the Problem

Although Annona senegalensis has been traditionally used for various medicinal purposes, scientific research on its effects against hepatocellular carcinoma and its antibacterial properties remains limited. The growing prevalence of hepatocellular carcinoma and the rising resistance of bacteria to conventional antibiotics highlight the urgent need for alternative therapeutic options. This study seeks to address this gap by evaluating the effects of methanol leaf extract of Annona senegalensis on hepatocellular carcinoma and its antibacterial potential.

Justification of the Problem

The exploration of Annona senegalensis for its hepatoprotective and antibacterial properties is crucial for several reasons. First, natural products often offer a safer and more affordable alternative to synthetic drugs. The antioxidant and anti-inflammatory properties of Annona senegalensis could play a significant role in mitigating the oxidative stress and inflammation associated with hepatocellular carcinoma (Nanti et al., 2024). Additionally, the antibacterial properties of the plant could provide a natural solution to combating antibiotic-resistant bacteria.

Understanding the effects of Annona senegalensis on liver biomarkers is essential for developing new therapeutic strategies for liver-related conditions. Liver non-enzyme markers, such as bilirubin, albumin, and total protein, provide critical information about liver function and the overall health of the liver. Studying these markers in the context of hepatocellular carcinoma can offer valuable insights into the disease 's progression and potential treatment options.

Aims and Objectives

The primary aim of this study is to investigate the effect of methanol leaves extract of Annona senegalensis on liver non-enzyme markers in DEN-induced hepatocellular carcinoma in rats. Additionally, the study aims to evaluate the antibacterial potential of the extract against common bacterial pathogens.

Specific Objectives:

1.To assess the impact of Annona senegalensis extract on liver non-enzyme markers in DEN-induced HCC in rats. This involves measuring liver function markers such as bilirubin, albumin, and total protein to determine the hepatoprotective effects of

the extract.

2.To evaluate the antibacterial activity of Annona senegalensis extract against selected bacterial strains. This involves testing the extract against common bacterial pathogens to determine its efficacy as an antibacterial agent.



Figure 1: Annona senegalensis

Ethnomedicinal Uses

All parts of A. senegalensis have been found useful for traditional medicine applications. The leaves have been used in treating yellow fever, tuberculosis, and small pox (Ofukwu et al., 2008; Okhale et al., 2016). The stem bark has been used in snakebite and hernia treatment (Dambatta and Aliyu, 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 (Okhale et al., 2016). 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 (Okhale et al., 2016). In Guinea, A. senegalensis has been employed in the treatment of malaria. In Swaziland, the bark is used to treat open sores (Okhale et al., 2016). Among the Igede people of Benue State in North Central Nigeria, the plant is used in combination with Ageratum conyzoides for diarrhoea and in

combination with Nauclea latifolia for dysentery (Igoli et al., 2005).

Use of A. senegalensis for pest control in Nigeria and Tanzania has also been documented. In the Republic of Benin, the fresh leaves are spread in poultry houses and left until they are dried. This is repeated once or twice a week for the control of parasites such as fleas and lice (Jada et al., 2014; Okhale et al., 2016).

Location	Local Name	Plant Part	Form	Conditions Treated
Nigeria	Abo/Abobo (Yoruba) Ubunu-ocha (Igbo) Gwandar-daji (Hausa)	Leaf	-	Yellow fever
Nigeria	Ukopko (Idoma)	Leaf, Root	Decoction	Tuberculosis
Nigeria	Gwandan daaji (Hausa) Dukuu-hi (Fulani)	Leaf	Infusion	Small pox
Nigeria	Gwandar daji (H)	Stem	-	Snake bites, Hernia
Cameroon	Doukkouhi ladde (Fulfulde)	Root	Infusion	Necrotizing Venoms
Nigeria	Gwandan daji (Hausa)	Root	Infusion	Erectile Dysfunction
Nigeria	Gwandan daaju (Hausa) Dukuu-hi (Fulani)	Root	Infusion	Difficulty in swallowing
Guinea	Sunsuningbe	Root bark	Decoction	Infectious diseases
Cameroon	Doukouhi	Root	Decoction	Gastritis, Snake bites, Male Sexual Impotence
Nigeria	Gwandan daji (Hausa)	Tree	Juice	Chicken pox
Republic of Congo	Moulolo (Kongo) Nlolo (Laadi) Ololo (Mbochi) Tchilolo (Vili)	Leaf, Seed, Bark	Decoction	Diabetes
Nigeria	Ahur/Anyam hul (Tiv)	Various	Various	Venomous bites

Phytochemistry

Phytochemical screening revealed the presence of various secondary metabolites including tannins, flavonoids, saponins, alkaloids, glycosides, steroids, volatile oil and anthocyanins. A. senegalensis has also been found to contain various minerals such as Ca, K, Mg, Zn, Fe, Cu, Mn, Pb, Cr as well as ascorbic acid and amino acids, making it an important source of nutrients (Okhale et al., 2016).

Essential oils

GC and GC–MS analyses showed p-cymene (36.0%), α-phellandrene (25.0%), α-pinene (8.3%), Z-sabinol (6.9%) and limonene (4.8%) as the major constituents of the essential oil of A. senegalensis stem bark (Khallouki et al., 2002). In another study, leaf

essential oil of A. senegalensis had oxygenated monoterpenes (65.0%) as the major constituents along with citronellal (30.0%), citronellol (14.8%), geranial (17.2%), thymol (8.1%), β–caryophyllene (7.8%) and carvacrol (6.92%) (Ameen et al., 2011). The essential oil, obtained by steam distillation of air-dried leaves of A. senegalensis growing in Burkina Faso was analyzed by GC and GCMS, the oil was found to contain germacrene D (19.2%), β-caryophyllene (19.1%), γ-cadinene (11.1%) and α-humulene (9.7%) as major components (Okhale et al., 2016).

In Brazzaville (Congo), essential oils were found in all parts of A. senegalensis including leaves, stem bark, root bark, epicarp and mesocarp (Nkounkou-Loumpangou et al., 2010). In Nigeria, nineteen monoterpenes and sesquiterpenes were identified in the essential oils of the leaves and fruits of A. senegalensis. The major constituents were car-3-ene in the fruit oil and linalool in the leaf volatile oil (Okhale et al., 2016).

Acetogenins

Annonaceous acetogenins are waxy substances consisting of C32 or C34 long chain fatty acids which have been combined with a propan-2-ol unit at C-2 to form a gama-lactone. They are only found in several genera of the plant family Annonaceae (Zeng et al., 1996). Two cytotoxic monotetrahydrofuran acetogenins, namely annogalene and annosenegalin (Fig. 2) have been isolated from the cytotoxic methanolic extract of A. senegalensis and A. cherimolia seeds. Their structures were established on the basis of 1D and 2D NMR spectroscopic techniques (Okhale et al., 2016).

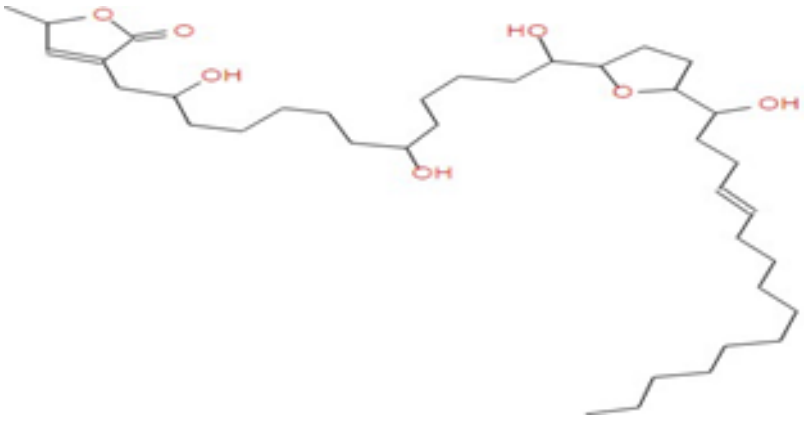


Figure 2: Structure of annogalene. Annosenegalin is 20-epimer, 23, 24-dihydro-annogalene (Okhale et al., 2016).

Alkaloids

Identification of alkaloids from A. senegalensis leaves had been reported. These included (-)-roemerine (Fig. 3), an aporphine alkaloid and (-)-isocorydine (Okhale et al., 2016).

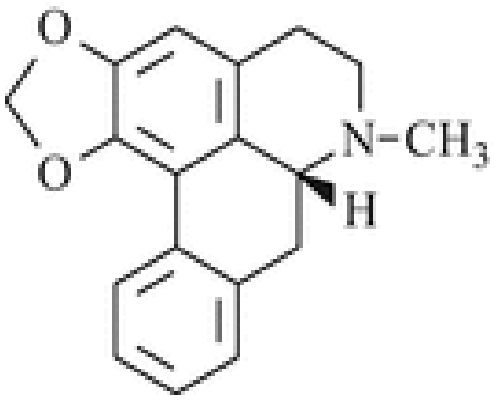


Figure 3: Structure of (-)-Roemerine (Okhale et al., 2016).

Biological Activities

Anticonvulsant Activity

Several studies have proven the anticonvulsant effect of A. senegalensis (Table 2). The plant is claimed in traditional medicine in Burkina Faso to be useful in the treatment of epilepsy. In a bid to validate these claims, Konate et al. (2012), studied the effect of aqueous extract on pilocarpine and picrotoxin induced seizures in mice and rats. Results showed that the plant extract 100 mg/kg and 150 mg/kg i.p had significant ($p<0.05$) curative effects on the convulsions induced by pilocarpine and at 50 mg/kg i.p had significant ($p<0.001$) preventive treatment effects on the convulsions induced by pilocarpine. Bioactivity-guided fractionation of the methanol-methylene chloride root bark extract of A. senegalensis using pentylenetetrazole (PTZ) induced seizures in mice resulted in the characterization of kaur-16-en-19-oic acid (KA), a diterpenoid. KA dose-dependently delayed onset of myoclonic spasms and tonic-clonic phases of PTZ-induced seizures and maximal electroshock seizures (Okhale et al., 2016).

Table 2: Anticonvulsant activity of A. senegalensis (Okhale et al., 2016).

Plant part	Test	Result
Aqueous extract of root bark	Pilocarpine-Induced Seizures in mice Picrotoxin-Induced Seizures in mice	Curative and preventive effects Protective effects
Methanol/Ethyl acetate extract of twigs/leaves	Pentylenetetrazole-induced convulsion in mice.	Delayed seizure onset; reduction in seizure duration; delayed time of death
Methanol extract of leaves	Pentylenetetrazole-induced convulsion in mice.	Non-dose-related inhibition of PTZ-induced seizures: increased seizure latency, prolonged duration of seizure and increased survival time
Methanol extract of root bark	Pentylenetetrazole-induced convulsion in albino mice.	Prolonged onset of tonic and clonic phases of seizure

Cytotoxic Activity

The in vitro cytotoxicity evaluation of methanol extract of the leaves was performed using A2780 ovarian cancer cells. A. senegalensis exhibited low cytotoxicity with an IC50 of

28.8µg/ml (Okhale et al., 2016). Similarly, the seeds have been tested against KB and VERO cell lines and bioassay-guided fractionation of seed extracts suggested that acetogenins were responsible for this activity (Saphaz et al., 1994). These have been identified as annosenegalin and annogalene in another study (Saphaz et al., 1996). Recently, kaurenoic acid from the root bark possessed antiproliferative effect against HeLa and PANC-1 cell lines (Okoye et al., 2014). Various fractions of the leaf extracts have showed mild to moderate cytotoxicity in different brine shrimp lethality bioassays (Okhale et al., 2016).

Antimicrobial Activity

In a study of methanol-methylene chloride extract of the root bark of A. senegalensis, the ethyl acetate fraction on further fractionation gave two active subfractions, a lipophilic oily and another fraction (AS2) which on purification precipitated white crystalline compound, later characterized to be kaurenoic acid (Okhale et al., 2016).

MICs of the ethyl acetate fraction, the lipophilic oily fraction and kaurenoic acid against Bacillus subtilis were 180, 60, and 30µg/ml respectively. AS2 exhibited activity against Staphylococcus aureus with an MIC of 150µg/ml, while the lipophilic oily fraction was active against Pseudomonas aeruginosa with an MIC of 40µg/ml. However, the extract and kaurenoic acid exhibited no effects against Candida albicans and Aspergillus niger. Ethanol extract of the plant had in vitro antimicrobial activity against some oral pathogens (Okhale et al., 2016). An extract of recipe containing six plants including A. senegalensis had significant antibacterial activity with (MIC) of 62.5µg/ml against S. aureus and 250µg/ml against C. albicans (Aiyeloja and Bello, 2006).

Table 3: Antimicrobial Activities of A. senegalensis (Source: Okhale et al., 2016).

Plant part	Test	Result
Methanol extract of Plant	in vitro activity against attenuated strains of Mycobacterium bovis (BCG).	MIC at 1250 µg/ml
Crude Tannins Isolated from the Leaf	Antibacterial activities against tested Bacteria	(MIC) gave 6.25 mg/ml on Shigella dysenteriae and 12.5 mg/ml on both Escherichia coli and Salmonella typhi.
Crude Flavonoids Isolated from Stem Bark	Antibacterial activities against tested Bacteria	(MIC) against Shigella specie and Escherichia coli at 100 mg/ml while against Salmonella typhi at 50 mg/ml
Crude extract of root bark	Antibacterial activities against tested Bacteria	MIC of methanol extract at 62.5 mcg/ml against S. aureus; No activity against E. coli and P. aeruginosa
Crude Extract from Stem Bark	Antibacterial activities against clinical isolates of bacteria	(MIC) of the methanol extract: 0.39mg/ml on E. coli, 3.17 mg/ml on S. enteriditis 25.00 mg/ml on S. dysenteriae.

Antiplasmodial Activity

The in vivo animal antimalarial activity of the methanol extract of A. senegalensis in mice was investigated. The extract at 100 mg/kg of mice gave 57.1% suppression of parasitemia. At doses of 800 mg/kg weight of mice, it induced the highest chemo suppression of parasitemia (91.1%) compared to chloroquine, which had a chemo suppression of 96.2%. The roots extract of A. senegalensis showed activity against the chloroquine resistant strain of Plasmodium falciparum (Okhale et al., 2016).

Antivenomous Activity

The potency of the methanol extract of the root bark of A. senegalensis was tested against cobra (Naja nigricotlis nigricotlis Wetch venom in rats. The extract caused reduction in the induced hyperthermia and directly detoxified the snake venom used by 16–33% (Okhale et al., 2016). It, however, failed to restore the biochemical functions (serum glutamate-oxalate-transaminase (sGOT) and serum-pyruvate-transaminase (sGPT)) of the liver (Adzu et al., 2005). In another study, a fraction of A. senegalensis leaf methanol extract neutralized lethal toxicity induced by Echis ocellatus venom. The key phytochemicals mediating the activity of this fraction were flavonoids and tannins (Okhale et al., 2016).

Spermatogenic Activity

Oladele et al. (2014), tested the aqueous leaf extract of A. senegalensis at different doses of 200, 300, and 500mg/kg body weight for its spermatogenic effect. Results showed the weight of the testes and epididymis increased significantly for the 300 and 500 mg/kg doses. The sperm concentration for the 200, 300 and 500 mg/kg doses also significantly increased and the sperm motility at 300 and 500 mg/kg also increased significantly. Decrease in abnormal sperm morphology was not significant for any of the doses. However, another study revealed that aqueous leaf extract of A. senegalensis may possess the potential to adversely affect testicular function in rat (Okhale et al., 2016).

Anti-Inflammatory and Analgesic Activities

In a bid to provide a scientific validation of the use of A. senegalensis against asthma and cough in the Ivorian Pharmacopoeia, Yeo et al. (2011), evaluated the anti-inflammatory effect of the ethanol extract of the leaf. It was observed that the extract induced a significant decrease in the number of inflammatory cells. Suleiman et al. (2014), studied the analgesic and anti-inflammatory properties of the methanol stem bark of A. senegalensis. The extract at 100, 400 and 1000 mg/kg orally, produced significant ($p<0.05$) dose dependent reduction in writhes induced by acetic acid, increased nociceptive reaction latency in hot plate test, caused significant ($p<0.05$) dose and time dependent decrease in the size of the paw oedema caused by egg albumin; and caused significant ($p<0.05$) inhibition of permeability of blue dye into the peritoneal cavity of mice induced by acetic acid. These results were comparable to those of standard drugs pentazocine and piroxicam (Okhale et al., 2016).

Hypnotic Activity

Effect of extract and fractions of A. senegalensis leaves on pentobarbitone-induced sleeping time was assessed. The extract and fractions significantly ($p<0.05$) shortened the sleep onset time (sleep latency) and prolonged sleeping time in a dose-related manner. A bioactive fraction significantly ($P<0.05$) prolonged sleep time but increased sleep latency in a dose-related manner (Okhale et al., 2016). Extracts of the root bark also potentiated the central nervous system depressant effect of phenobarbitone in a dose dependent fashion (Okhale et al., 2016).

Antioxidant Activity

In a study designed to investigate the antioxidant and drug detoxification potential of A. senegalensis leaf, aqueous extract of A. senegalensis leaf scavenged free radicals in vitro and reversed CCl_4-induced hepatocellular damage in rats (Ajboye et al., et al., 2010). Extract from the leaf of A. senegalensis from Burkina Faso had an improved antioxidant activity compared to that from Togo. This may be due to higher polyphenolic flavonoids in those from Burkina Faso (Okhale et al., 2016).

Anthelmintic Activity

Ethanol leaf extract of A. senegalensis was assessed for egg-inhibition ability as well as toxicity against the infective larval stage of Haemonchus contortus. The extract at 0.8%w/v caused a 98.33% mortality of the larva but had no effects on egg hatching (Okhale et al., 2016). In another study however, where whole ground plant material was used, the extract of A. senegalensis showed significant ($P<0.001$) reduction in egg hatch at a concentration of 7.1 mg/ml. (Alawa et al., 2003). The root of Annona senegalensis showed anthelminthic activity. Five acetogenins namely gigantetronenine, squamocine, glaucanisine, glaucanetine, goniothalamicine and two alkaloids liriodenine and norolivéroline were isolated and characterized. Squamocine was more potent than levamisole, the reference substance (Okhale et al., 2016).

Antitrypanosomal Activity

Several studies have proven that A. senegalensis has potent in vivo trypanocidal effects, with little or no in vitro effects (Okhale et al., 2016). Further investigations have shown that these activities are due to isolated acetogenins from extracts of the seeds. Acetogenins probably responsible for this activity are annogalene, annonacin, annonacin A,

annosenegalin and senegalene (Okhale et al., 2016).

Insecticidal Activity

Several studies have shown insecticidal properties of A. senegalensis. An aporphine alkaloid, (-)-roemerine was identified as active principle responsible for the insecticidal activity (Okhale et al., 2016).

Haemostatic Activity

A. senegalensis is sold by herbalists in South Benin for treatment of bleeding. Dougnon et al. (2012), in a bid to find scientific evidence for this use, performed in vitro haemostatic tests on hydro alcoholic extracts of the leaves. Results confirmed its anticoagulant properties, as indicated by a 39% reduction of plasma re-calcification time. A. senegalensis was also shown to have astringent property, so it could act on primary haemostasis through vasoconstriction (Okhale et al., 2016).

Hepatocellular (HCC)

Hepatocellular carcinoma (HCC) has become the most common primary hepatic malignancy, with average survival rates between 6 and 20 mo (Waller et al., 2015). It now ranks sixth in the world among all malignancies, contributing to the third leading cause of mortality attributed to cancer (Bravi et al., 2013). Incidence worldwide has increased, likely due to the rising incidence of chronic hepatitis B and C infections. Since 1963 when first performed by Starzl et al. (1963), liver transplantation has seen dramatic changes, though initial outcomes were suboptimal. Attempts to treat HCC with liver transplantation showed poor results. At this point, it was determined that a narrow spectrum of selection criteria was needed to increase survival during the time after transplant. In 1996, Mazzaferro et al (1996), in his revolutionary paper, proposed stricter criteria for liver transplantation. The four-year rate of survival was 75% with an 83% survival rate without recurrence (Waller et al., 2015). From this landmark study, the Milan Criteria (MC) was established. The MC includes three major points: an isolated malignancy $\leq$ 5 cm, or 2-3 tumors each $<$ 3 cm, that does not have any evidence of invasion into the vascular system or dissemination outside the liver (Waller et al., 2015). The MC became accepted for assessing individuals that have HCC as candidates for transplantation (Lavanchy, 2004). Given the high mortality associated with HCC, there has been a recent discussion on expanding the current criteria to include more patients as potential transplant candidates, and, therefore, increase overall survival (Waller et al., 2015).

In the hopes of improving disease-free survival, there may be certain ways to help incorporate more candidates with HCC. These may include expanding the current Milan and University of California San Francisco (UCSF) criteria to include tumor markers and histology, increasing the number of living donor transplants for HCC, using sorafenib post-transplant, and utilizing alternative immunosuppressive regimens (Waller et al., 2015).

Etiology

Worldwide, chronic hepatitis B contributes to the greatest number of HCC. Chronic hepatitis C is primarily the cause in Southern Europe and North America. Individuals that have chronic hepatitis B may develop HCC without evidence of cirrhosis (Lavanchy, 2004). However, 70%-90% of patients suffer from concurrent cirrhosis (Waller et al., 2015). Some factors, such as elevated viral loads, and having hepatitis B envelope and surface antigens are believed to contribute to HCC incidence (Waller et al., 2015). Advanced age, being male, obesity, alcohol abuse, diabetic, and family history, are variables associated with increased risks for developing HCC. Hepatitis B and C co-infection have a cumulative effect in contributing to the formation of HCC. Additional variables of risk for HCC are in Table 4. The United States, as well as other developed countries, have increasingly seen non-alcoholic steatohepatitis (NASH) as a primary contributor. It is assumed that the obesity epidemic and prevalence of diabetes has played a significant role. Associated factors include Age, male gender, hepatitis C virus (HCV)/hepatitis B virus, alcohol abuse, severity of non-alcoholic fatty liver disease/ NASH, diabetes/obesity, iron overload, and genetic variants (PNPLA3, APOB, TERT) (Waller et al., 2015).

MATERIALS AND METHODS

Materials

Apparatus

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

Reagents

Diethyl nitrosamine (DEN), Silymarin Standard, EDTA Bottles, Ethanol

Methods

Preparation of Plant Extract

The collected plant materials were washed, sliced and completely shaded 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 liter) in an airtight 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 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) male albino rats (weighing between 140 ±20 g) were obtained from HAUEMM Veterinary Animal House, Federal Housing Estate, Yola, 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. The guide for the Care and Use of Laboratory Animals was strictly followed.

Experimental Design

The rats were 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.). The methanol extract, was administered to the rats through oral gavages for a period of 14 days.

Group Treatment

I (Normal control) Normal + No treatment

II (Negative control) DEN + No treatment

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

IV (Treatment I) DEN + Methanol Extract (200 mg/kg bw)

V (Treatment II) DEN + Methanol Extract (400 mg/kg bw)

VI (Treatment III) DEN + Methanol Extract (600 mg/kg bw)

Collection of Samples

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

Estimation of Non-Enzyme Liver marker

The blood samples were taken from the heart of the rats and centrifuged for five minutes at 3000 rpm to prepare the serum for biochemical analysis. The TB (total bilirubin), DB (direct bilirubin), TP (total protein) and ALB (albumin) levels were determined by the method adopted by Umaru et al. (2024)

Antibacterial Assay

The antibacterial activity of methanolic leaf extract of Annona senegalensis was determined by the agar well diffusion method as reported by Umaru et al. 2022. The 20 mL of molten nutrient agar was poured into each of the Petri dishes and allowed to solidify. The 0.5 McFarland standardized bacterial broth, was spread on the dry nutrient agar with the aid of a spreader pre-sterilized in ethanol and flame overnight. With the aid of a sterile cork-borer, four wells of 6 mm in depth each and about 5 cm apart, were made in the nutrient agar. Three of the wells were filled with 500 µL of the Annona senegalensis leaf extract dissolved in sterile distilled water, one well with the water only (the negative control) and the last with 1% standard antibiotic, gentamicin. The positive control was dispensed into the wells in triplicates. After incubating for 24 hrs at 37°C, the antibacterial activities were determined by measuring the diameter of the inhibition zone. The zones of inhibition observed with the extract were compared with that of the standard antibiotic, chloramphenicol. The measured chloramphenicol inhibition zones 'diameters were subsequently matched with the respective standard zones 'diameters for Escherichia coli

(Gram-ve), Staphylococcus aureus, (Gram +ve), Bacillus subtilis (Gram +ve) and Pseudomonas aeruginosa (Gram-ve). The Annona senegalensis zone of inhibition from 9-14 mm in diameter was taken as a positive antibacterial activity based on the growth inhibition standard as reported by Umaru et al. 2022.

RESULTS

Table 4: Results for Liver Function Test

Treatments	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	ALB (g/L)	TP(g/L)	DB(mmol/L)	TB (mmol/L)
Normal Control	91.59 ± 0.59^{a}	25.69 ± 1.52^{a}	142.25 ± 0.66^{a}	6.05 ± 0.25^{a}	7.33 ± 0.60^{a}	0.94 ± 0.06^{a}	1.83 ± 0.12^{abc}
Negative Control	69.52 ± 0.88^{b}	54.97 ± 1.54^{b}	368.70 ± 1.14^{b}	1.33 ± 0.26^{b}	2.60 ± 0.59^{b}	0.93 ± 0.06 ^{a}	2.07 ± 0.23^{b}
Positive Control	63.50 ± 1.00^{c}	17.40 ± 1.08^{c}	155.32 ± 1.41^{c}	4.84 ± 0.42^{c}	7.70 ± 0.35^{a}	0.94 ± 0.07^{a}	2.03 ± 0.15^{b}
Treatment 1	65.61 ± 2.03^{c}	19.62 ± 0.52^{d}	256.27 ± 1.13^{d}	4.52±0.22^{ce}	7.20 ± 0.25^{a}	1.04 ± 0.06 ^{b}	1.90 ± 0.03^{bc}
Treatment 2	48.71 ± 1.21^{d}	20.74 ± 1.06^{d}	271.61 ± 1.06^{e}	5.11 ± 0.10^{d}	6.37 ± 0.13^{c}	1.13 ± 0.03^{a}	1.63 ± 0.05^{a}
Treatment 3	65.40 ± 1.01^{c}	18.71 ± 1.00^{cd}	274.80 ± 1.26^{f}	4.32 ± 0.18^{e}	5.70 ± 0.13^{c}	0.96 ± 0.08^{a}	1.71 ± 0.05^{ac}

Results are expressed in Mean ± standard deviation. Value with same superscript within a column are statistically not significant while values different superscript within a column are statistically significant ($P<0.05$)

Normal Control = Normal feed (no treatment), Negative control = DEN (50mg/kg b.w) + No treatment, Positive control = DEN (50mg/kg b.w) + Standard Drug (Silymarin 100 mg/kg bw), Treatment 1 = DEN (50mg/kg b.w) + A. Senegalese Extract (200 mg/kg bw), Treatment 2 = DEN (50mg/kg b.w) + A. senegalese Extract (400 mg/kg bw), Treatment 3 = DEN (50mg/kg b.w) + A. senegalese Extract (600 mg/kg bw)

DISCUSSION

The liver function test presented in the results section provides insights into the effects of different treatments on various, the effects of different treatments on key biochemical markers such as AST, ALT, ALP, albumin, total protein, direct bilirubin, and total bilirubin. The normal control group exhibited stable and healthy levels across all parameters, while the negative control group showed significant deviations, indicating liver

damage or dysfunction. The positive control, as expected, demonstrated an improvement in most markers, showing its potential in restoring liver function.

Among the treatments, Treatment 2 stood out with the lowest AST (48.71 IU/L) and TB (1.63 mmol/L), suggesting it was the most effective in reducing liver stress. It also had the highest albumin level (5.11 g/L), which indicates better liver protein synthesis. However, its ALP level (271.61 IU/L) was still higher than the positive control, suggesting it may not be as effective in reducing bile duct-related issues. Treatment 1 also performed well, with total protein levels (7.20 g/L) close to normal, indicating that it helped maintain protein metabolism.

Treatment 3 had a moderate effect compared to the others. While it showed improvement in ALT (18.71 IU/L) and TB (1.71 mmol/L), its total protein (5.70 g/L) was the lowest among the treatments, suggesting a less favorable impact on protein synthesis. Additionally, its ALP level (274.80 IU/L) was the highest among the treatments, which may indicate some residual liver stress or bile duct dysfunction.

Comparatively, all treatments showed some level of hepatoprotective effect compared to the negative control, as they helped reduce ALT, AST, and bilirubin levels. However, Treatment 2 demonstrated the most promising results in terms of liver enzyme reduction and protein synthesis improvement, though its ALP levels suggest that further investigation is needed to assess its overall efficacy.

CONCLUSION

These findings suggest that Treatment 2 could be the most effective option for liver protection, followed closely by Treatment 1. However, Treatment 3 showed a moderate effect and may not be as effective as the other two. Future studies should explore the mechanisms behind the differences in ALP levels among treatments and determine whether Treatment 2 can be optimized for better overall liver health.

Recommendation

1.Further studies should investigate the mechanism of action behind the differences in ALP levels and explore potential optimization of the most effective treatment to ensure comprehensive liver protection.

2.Further studies should investigate the underlying mechanism and explore ways to optimize its formulation to improve ALP regulation.

3.Since all treatments resulted in ALP levels higher than the positive control, further research should focus on understanding whether this indicates residual bile duct stress or an unintended effect of the treatments. Adjustments in dosage or combination with bile-supportive agents may be explored.

4.Future studies should include long-term observations to evaluate the sustained effectiveness and safety of the treatments. Additionally, in vivo studies with different dosages should be conducted to determine the most effective and safe administration strategy.

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