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EXPLORING THE ANTICANCER POTENTIAL OF CLINACANTHUS NUTANS ETHANOLIC LEAF EXTRACT IN DIETHYLNITROSAMINE-INDUCED HEPATOCELLULAR CARCINOMA IN WISTAR ALBINO RATS: A COMPREHENSIVE REVIEW

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ABSTRACT

Hepatocellular carcinoma (HCC) remains a leading cause of cancer-related morbidity and mortality worldwide, particularly in regions with high exposure to environmental carcinogens and chronic liver diseases. Conventional treatment modalities such as chemotherapy and surgical resection are often limited by adverse effects, poor accessibility, and high recurrence rates. Emerging evidence suggests that oxidative stress and chronic inflammation play pivotal roles in the pathogenesis and progression of HCC, thereby underscoring the therapeutic relevance of antioxidant-based interventions.In this context, *Clinacanthus nutans*, a medicinal plant renowned in traditional Southeast Asian medicine, has garnered interest due to its potent antioxidant, anti-inflammatory, and cytoprotective properties. This comprehensive study evaluates the chemopreventive efficacy of ethanolic extract of *C. nutans* leaves (CCNEE) in a well-established rat model of HCC induced by diethylnitrosamine (DEN) and carbon tetrachloride (CCl_4). Experimental groups received CCNEE at two dose levels (200 mg/kg and 400 mg/kg body weight), with silymarin (100 mg/kg) serving as a standard comparator. Biochemical assessments revealed a significant reduction in liver enzymes (AST, ALT, ALP), bilirubin levels, and lipid peroxidation, along with restoration of total protein and cholesterol levels. Hematological parameters, including hemoglobin concentration, red and white blood cell counts, and differential leukocyte profiles, were markedly improved. Moreover, antioxidant enzyme activities (SOD, CAT) were significantly elevated in CCNEE-treated groups, indicating amelioration of oxidative stress. Histopathological analysis corroborated these findings, showing preserved hepatic architecture and reduced tumor burden in treated animals, particularly at the higher dose.

INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for approximately 75–90% of primary liver cancers and represents the third leading cause of cancer-related deaths globally. In India, about 1.1 million new cancer cases are diagnosed annually, with a rising trend attributed to lifestyle factors, viral infections, and environmental carcinogens. The standard treatments for HCC—surgical resection, chemotherapy, and transplantation — often suffer from

limitations such as toxicity, resistance, and high recurrence rates. In this context, natural products and plant-based therapies have drawn increasing interest. Medicinal plants offer a diverse reservoir of bioactive compounds, many of which exhibit potent anticancer, antioxidant, and anti-inflammatory activities. *Clinacanthus nutans*, a member of the Acanthaceae family, is traditionally used across Southeast Asia for ailments ranging from fever and infections to tumors. This review consolidates in-depth experimental findings demonstrating the efficacy of its ethanolic extract (CCNEE) against chemically induced HCC in Wistar albino rats.

Pathophysiological Basis of HCC and Rationale for DEN-Induced Model:

HCC typically arises on a background of chronic liver inflammation, fibrosis, and cirrhosis. Reactive oxygen species (ROS), released during chronic inflammation or chemical insult, initiate lipid peroxidation, oxidative DNA damage, and activation of oncogenic pathways. Diethylnitrosamine (DEN), a well-characterized nitrosamine, exerts its carcinogenic effect through cytochrome P450-mediated bioactivation, leading to DNA adduct formation and mutagenesis. Its co-administration with CCl_4 enhances oxidative liver injury, providing a reproducible experimental model to evaluate chemopreventive strategies.

This DEN+CCl_4 model closely mimics human hepatocarcinogenesis in terms of etiology, progression, and molecular signatures, making it an ideal platform for preclinical evaluation of hepatoprotective agents, Ethnopharmacology and Phytochemistry of Clinacanthus nutans:

Traditional Uses:

Clinacanthus nutans, locally known as Sabah snake grass or Dandang Gendis, is widely used in Malaysia, Indonesia, Thailand, and India for its anti-inflammatory, anti-viral, and wound-healing properties. Ethnobotanical surveys report its use in treating herpes simplex, skin lesions, and chronic inflammation—conditions that share oxidative stress as a common pathology.

Phytochemical Constituents:

Phytochemical screening of CCNEE revealed the presence of:

- **Flavonoids**: Potent antioxidants capable of scavenging superoxide and hydroxyl radicals.
- **Alkaloids and phenolics**: Known for their cytotoxic and chemo preventive properties.
- **Saponins, glycosides, and tannins**: Contribute to anti-inflammatory and hepatoprotective actions.
- **Steroids**: Involved in membrane stabilization and enzyme modulation.

These constituents are believed to act synergistically, mitigating hepatocellular damage and suppressing carcinogenesis.

Methodological Synopsis of Experimental Design: The study was conducted on 30 male Wistar rats randomized into five groups:

1. **Normal Control** – received normal saline.
2. **HCC Control** – received DEN (100 mg/kg i.p. weekly ×3 weeks) followed by CCl_4 (3 mL/kg s.c. for 2 weeks).
3. **Standard Group** – HCC induced + silymarin (100 mg/kg i.p.).
4. **Test Group 1** – HCC induced + CCNEE 200 mg/kg (p.o.).
5. **Test Group 2** – HCC induced + CCNEE 400 mg/kg (p.o.).

Biochemical parameters were evaluated using spectrophotometric assays. Hematological indices were assessed via hemocytometry and histological analysis employed hematoxylin-eosin staining. Ethical approvals followed CPCSEA and IAEC protocols.

RESULTS:

1. Body and Liver Weight: DEN (diethylnitrosamine) and CCl_4 administration significantly reduced overall body weight by inducing systemic toxicity and impaired appetite—both common signs in tumor-bearing animals. Liver weights increased markedly due to hepatomegaly, nodular proliferation, and inflammation. Treatment with CCNEE (200 and 400 mg/kg) and silymarin reversed these trends,

with the higher dose of CCNEE showing comparable efficacy to the standard. This suggests a protective or regenerative effect on hepatic tissue.

2. Hematological Parameters

DEN-intoxicated animals exhibited hallmark features of hepatic dysfunction:

- **Anemia** (↓ hemoglobin, ↓ RBC): Indicative of impaired erythropoiesis or blood loss from liver pathology.
- **Leukocytosis** (↑ WBC, neutrophils): Suggests systemic inflammation and immune activation.
- **Lymphocytosis**: Possibly a response to abnormal hepatocyte proliferation and cytokine release.

Post-treatment with CCNEE restored these values close to normal. The reversal implies correction of hepatic-induced hematotoxicity, likely due to the antioxidant and anti-inflammatory properties of the extract's flavonoids and alkaloids.

3. Liver Function Enzymes and Metabolic Biomarkers

DEN+CCl_4 significantly elevated:

- **SGOT/AST, SGPT/ALT**: Markers of hepatocellular damage.
- **ALP**: Reflects biliary obstruction and cholestasis.
- **Total Bilirubin**: Elevated levels are linked to compromised bilirubin metabolism and excretion.

Concomitantly, there was:

- **Reduction in Total Protein**: Impaired protein synthesis in hepatocytes.
- **Rise in Serum Cholesterol**: Linked to disrupted lipid homeostasis.

Treatment with CCNEE resulted in:

- Restoration of transaminase levels, suggesting membrane stabilization and reduced leakage of cytosolic enzymes.
- Normalization of total protein and cholesterol, indicating functional recovery of synthetic and metabolic pathways.

Notably, the 400 mg/kg dose had effects nearly identical to silymarin, underscoring the dose-response relationship.

4. Tumor Burden Assessment

DEN induced an average of 68 hepatic nodules per liver, with volumes averaging 4.2 mm—consistent with rapid, multifocal tumor growth. CCNEE administration, especially at 400 mg/kg:

- Reduced nodule count to 21.
- Diminished nodule volume to 1.9 mm.

This dramatic reduction in tumor burden demonstrates the antitumorigenic properties of *C. nutans*, likely due to inhibition of cell proliferation and promotion of apoptotic pathways.

5. Oxidative Stress and Antioxidant Enzymes

DEN+CCl_4 elevated **lipid peroxidation (LPO)**, a marker for ROS-induced membrane damage. Simultaneously, **SOD and CAT** levels decreased, indicating depletion of antioxidant defenses. CCNEE treatment:

- Significantly enhanced SOD and CAT activity.
- Suppressed LPO accumulation.

This confirms that CCNEE reestablishes oxidative balance, protecting hepatocytes from free radical injury—a key mechanism in halting carcinogenesis.

6. Histopathological Insights

Liver sections from HCC control rats showed:

- Severe necrosis.
- Mononuclear inflammatory infiltration.
- Cytoplasmic ballooning.
- Disrupted lobular architecture.

By contrast, CCNEE-treated groups revealed:

- Restoration of sinusoidal spaces.
- Reappearance of hepatic cords.
- Reduced inflammation and fatty changes.

High-dose CCNEE closely resembled the hepatoprotective pattern of silymarin.

DISCUSSION:

The efficacy of *C. nutans* ethanolic leaf extract in combating DEN-induced hepatocarcinogenesis is multifaceted:

A. Hepatic Recovery and Membrane Integrity: The extract restored enzyme levels and improved histological architecture, suggesting it reinforces hepatocyte membranes. This prevents enzyme leakage and promotes functional enzyme retention in hepatocytes—critical for detoxification and metabolism.

B. Antioxidant Mechanism: The balance between ROS and endogenous antioxidants is central to liver health. DEN overwhelms this balance, resulting in oxidative DNA damage and lipid peroxidation. Flavonoids and phenolics in CCNEE likely donate electrons to neutralize radicals, upregulate antioxidant enzymes (SOD, CAT), and inhibit propagation of oxidative chains.

C. Immune Modulation and Hematopoiesis: Leukocyte indices—neutrophils and lymphocytes—are indicators of inflammatory signaling and immune surveillance. Restoration of these parameters following CCNEE treatment suggests immunomodulatory action, possibly via downregulation of TNF-α or IL-6 signaling, which are commonly elevated in DEN models.

D. Antitumor Effects: The reduction in tumor nodule number and size indicates the extract's ability to:

- Inhibit cellular proliferation.
- Promote apoptotic clearance of pre-neoplastic cells.
- Arrest cell cycle progression.

This may be mediated through downregulation of MAPK, NF-κB, or p53-mutant signaling—a hypothesis warranting further investigation through molecular assays.

E. Synergism of Phytoconstituents: The overall effect is not attributable to one compound but rather the synergistic interplay among:

- **Flavonoids** (e.g., apigenin, luteolin): antioxidant, anti-inflammatory.
- **Alkaloids**: cytotoxic, DNA intercalation.
- **Tannins/saponins**: membrane active, free radical scavenging. Polypharmacy effect mirrors the holistic benefit observed in traditional medicine.

F. Comparison with Silymarin: Silymarin, a benchmark hepatoprotective agent, shares flavonolignan-based antioxidant activity. CCNEE's comparable efficacy suggests it could be used as a plant-based alternative or adjunct, with possible advantages in avai lability and cost.

Parameter	HCC Control	CCNEE 200 mg/kg	CCNEE 400 mg/kg	Silymarin 100 mg/kg
Liver Enzymes (ALT, AST, ALP)	↑↑	↓	↓↓	↓↓
Lipid Peroxidation (LPO)	↑↑	↓	↓↓	↓↓
SOD, CAT	↓↓	↑	↑↑	↑↑
Tumor Nodules (avg count)	68 ± 5	28 ± 4	21 ± 5	18 ± 4
Histopathology	Severe necrosis	Mild improvement	Near-normal	Normal

CONCLUSION:

The present investigation convincingly demonstrates that the ethanolic extract of *Clinacanthus nutans* leaves (CCNEE) exerts potent hepatoprotective and anticancer effects in a chemically induced model of hepatocellular carcinoma (HCC) in Wistar albino rats. The administration of diethylnitrosamine (DEN) and carbon tetrachloride (CCl_4) resulted in profound hepatic dysfunction, manifested by elevated liver enzymes, hematological dysregulation, oxidative stress, and histopathological alterations. These pathological features were significantly ameliorated upon treatment with CCNEE, with the higher dose (400 mg/kg) yielding outcomes comparable to the standard hepatoprotective agent, silymarin. The extract not only restored hepatic architecture and biochemical parameters but also markedly reduced tumor burden, highlighting its potential antiproliferative and antioxidant efficacy. The phytoconstituents identified—such as flavonoids, alkaloids, and phenolic compounds—likely underpin the observed therapeutic benefits, acting through mechanisms that counteract free radical damage, modulate immune responses, and stabilize cellular membranes.

Collectively, these findings substantiate the traditional use of *Clinacanthus nutans* and pave the way for its development as a complementary or alternative therapy for liver cancer. Future work should focus on isolating its bioactive constituents, elucidating molecular targets, and evaluating long-term safety and efficacy through advanced preclinical and clinical studies.

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