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HEPATOPROTECTIVE POTENTIAL OF ACTINIDIA DELICIOSA AGAINST CARBON TETRACHLORIDE-INDUCED LIVER INJURY IN ALBINO RATS

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Abstract
This research examines the protective capabilities of alcoholic extract derived from Actinidia deliciosa (kiwi fruit) against hepatic damage induced by carbon tetrachloride (CCl_4) in albino rats. The investigation incorporated biochemical parameter analysis and histopathological examination to evaluate the extract's hepatoprotective efficacy. Male albino rats were organized into distinct experimental groups and subjected to CCl_4 exposure to induce liver injury, with concurrent administration of either Actinidia deliciosa extract or standard hepatoprotective agent silymarin. The study assessed critical hepatic markers, including serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase (ALP), and total bilirubin. Results demonstrated that ethanolic extract of Actinidia deliciosa significantly ameliorated CCl_4-induced alterations in biochemical parameters, with the lower dose (200 mg/kg) exhibiting greater efficacy than higher doses (400 mg/kg), and in some instances surpassing the standard reference drug silymarin. The extract also displayed substantial antioxidant activity in DPPH radical scavenging assays, with an IC_{50} value of 305 µg/mL compared to silymarin's 585 µg/mL. Histopathological examination supported these findings, showing preservation of normal hepatic architecture in extract-treated specimens. This investigation provides compelling evidence supporting the potential therapeutic application of Actinidia deliciosa in managing hepatic disorders characterized by oxidative damage.

Keywords: Actinidia deliciosa, carbon tetrachloride, hepatoprotective, oxidative stress, liver enzymes, antioxidant activity

Introduction

1.1 Historical Context of Herbal Medicine

Throughout the early 1900s, botanical preparations constituted the foundation of healthcare systems worldwide, primarily due to the absence of pharmaceutical agents such as analgesics and antibiotics. The subsequent emergence and rapid advancement of modern allopathic medicine precipitated a decline in herbal therapy utilization, largely attributable to the expeditious action of chemically synthesized medications. However, over successive decades, the limitations and adverse effects associated with conventional pharmaceutical interventions became increasingly apparent. Recently, a

resurgence in plant-based therapeutic approaches has been observed, with certain natural products demonstrating comparable efficacy to their synthetic counterparts (Singh, 2007).

This renaissance in herbal medicine underscores the importance of developing comprehensive understanding across various disciplines including Ayurveda, plant taxonomy, medicinal botany, phytochemical analysis, metabolic biochemistry, ethnopharmacology, and safety pharmacology—knowledge domains crucial for practitioners of traditional healing systems. The global herbal pharmaceutical sector has experienced exponential growth in recent years, with epidemiological surveys confirming increasing patient preference for traditional healers and remedies. Notably, the World Health Organization has acknowledged the critical role of herbal therapeutics in contemporary healthcare. According to research conducted in the United States, approximately 60-70% of individuals in rural communities depend on traditional plant remedies for addressing common ailments (Singh, 2007).

2.1 Liver Diseases and Conventional Treatment Limitations

The liver represents a vital organ performing numerous physiological functions including detoxification of harmful compounds, protein synthesis, and production of biochemicals essential for digestive processes. Additionally, it contributes to metabolism, glycogen storage, red blood cell recycling, and hormone regulation. Given its central role in metabolism and detoxification, the liver remains particularly vulnerable to injury from various toxins, drugs, and infectious agents.

Conventional pharmaceutical interventions for liver disorders often present limitations including adverse effects, contraindications in certain patient populations, and variable efficacy across different hepatic pathologies. These constraints have stimulated research interest in alternative therapeutic approaches, particularly plant-based remedies with established traditional usage history and favourable safety profiles.

1.1 Botanical Hepatoprotective Agents

Botanical medicines constitute a rich reservoir for hepatoprotective treatments. Both single-herb and multi-herb preparations are currently employed therapeutically for various hepatic conditions. Research indicates that more than 700 different herbal formulations derived from over 100 botanical sources are administered in various forms including decoctions, tinctures, pills, and capsules. However, emerging scientific literature also documents instances where herbs previously considered liver-friendly have demonstrated potential hepatotoxicity, underscoring the importance of rigorous scientific evaluation of traditional remedies.

Milk thistle (Silybum marianum) has long been recognized for its liver-protective properties and remains a benchmark in hepatological care. In India, Picrorhiza kurroa reportedly demonstrated superior hepatoprotective efficacy compared to milk thistle, though these findings were inconclusive and lacked comprehensive validation (Singh, 2007). Additional botanicals documented for their potential liver-protective effects include Eclipta alba (false daisy), Glycyrrhiza glabra (liquorice), Boerhaavia diffusa (punarnava), Phyllanthus amarus (stonebreaker), Uncaria gambir (gambir), and Andrographis paniculata (kalmegh).

Actinidia deliciosa as a Potential Hepatoprotective Agent

Actinidia deliciosa, commonly known as kiwi fruit, represents a botanical specimen that has received limited research attention regarding its potential hepatoprotective properties despite its rich nutritional profile and traditional medicinal applications. The fruit contains significant concentrations of vitamins C and E, polyphenols, and other bioactive compounds with demonstrated antioxidant properties—characteristics potentially beneficial for mitigating oxidative stress-induced hepatic damage.

The present research investigates the hepatoprotective potential of alcoholic extract derived from Actinidia deliciosa against carbon tetrachloride-induced liver injury in albino rats. Carbon tetrachloride represents a well-established hepatotoxin utilized extensively in preclinical models to induce predictable liver damage characterized by oxidative stress, lipid peroxidation, and subsequent hepatocellular necrosis.

Materials and Methods

Plant Material and Extract Preparation

Fresh specimens of Actinidia deliciosa were sourced from authenticated suppliers, thoroughly cleaned, and subjected to systematic botanical identification. The plant material underwent careful processing, including drying under controlled conditions to preserve bioactive constituents. The dried material was subsequently pulverized to achieve uniform particle size, facilitating efficient extraction. The alcoholic extract was prepared using ethanol as the extraction solvent in a Soxhlet apparatus. Extraction proceeded until complete exhaustion of the plant material, as evidenced by colourless solvent in the extraction chamber. The resulting extract underwent concentration under reduced pressure using a rotary evaporator, followed by complete drying to yield a solid residue. This ethanol extract of Actinidia deliciosa (EEAD) was preserved in airtight containers under refrigeration until experimental utilization.

Phytochemical Analysis

Preliminary phytochemical screening was conducted on EEAD to determine the presence of major phytoconstituent classes, including alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides, following standard procedures. Additionally, physicochemical evaluations including ash content, extractive values, and moisture determination were performed according to established pharmacopeial methods.

2.3 Determination of Ash Content

Ash content analysis was performed to determine the presence of inorganic substances such as carbonates, silicates, oxalates, and phosphates. This parameter provides valuable information regarding drug purity and potential adulteration. Approximately 2 grams of dried plant material were precisely measured using pre-weighed silica containers and subjected to ignition at temperatures below 450°C until complete elimination of carbonaceous matter. Upon cooling, the residue was weighed, and ash percentage was calculated relative to the dried sample weight.

Water-soluble ash was determined by boiling the total ash with 25 mL purified water for five minutes. The insoluble fraction was collected, rinsed, ignited at 450°C for fifteen minutes, and weighed. Water-soluble ash was calculated by subtracting insoluble material weight from total ash weight, expressed as a percentage of the dried sample.

Acid-insoluble ash was estimated by heating the ash with 25 mL of 2M hydrochloric acid for fifteen minutes. Undissolved solids were collected, washed with warm water, ignited, cooled, and weighed. The acid-insoluble residue proportion was calculated relative to the air-dried herbal material.

2.4 Extractive Value Determination

Extractive values approximate the quantity of bioactive substances soluble in defined solvents. Sequential extraction using solvents of increasing polarity (petroleum ether, ethanol, and water) was performed to obtain comprehensive extractive profiles.

For water-soluble extractive determination, five grams of powdered plant material were soaked in 100 mL distilled water in a sealed container for 24 hours, with intermittent agitation during the initial six hours. Following filtration, 25 mL of filtrate was evaporated to dryness at 105°C, and the residue was weighed. The extractive percentage was calculated based on the dried sample weight.

Similarly, alcohol-soluble extractive was determined by immersing 5 grams of powdered sample in 100 mL of ethanol. After filtration, 25 mL of filtrate was evaporated at 105°C to dryness and weighed. The proportion of alcohol-soluble extractive was computed as a percentage of the dried plant material.

Antioxidant Activity Assessment

The antioxidant potential of EEAD was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Various concentrations of extract (100-1000 µg/mL) were prepared in methanol. To 1 mL of each concentration, 3 mL of methanolic DPPH solution (0.1 mM) was added. The mixtures were vortexed for 15 seconds and incubated at 37°C for 30 minutes. Absorbance

reductions at 517 nm were measured every 15 minutes at ambient temperature using a spectrophotometer. Silymarin served as the reference standard. All experiments were conducted in triplicate. The percentage of DPPH radical inhibition was calculated using the formula:
DPPH inhibition (%) = [(Abs normal - Abs trial) / Abs normal] × 100
Where:
- Abs normal = absorbance of the normal at time zero
- Abs trial = absorbance of the test sample after 15 minutes

The concentration producing 50% inhibition (IC_{50}) was determined for both EEAD and silymarin.

Acute Toxicity Study
Acute toxicity evaluation of EEAD was conducted according to OECD guidelines to establish safety parameters and determine appropriate dosing for subsequent efficacy studies. Healthy adult albino rats weighing 150-200 grams were utilized. Following acclimatization, animals were fasted overnight prior to extract administration. EEAD was administered orally in escalating doses, beginning with 400 mg/kg and increasing up to 4000 mg/kg body weight. Animals were observed continuously for the first four hours and subsequently at 24-hour intervals for 14 days. Observations included mortality, behavioral changes, autonomic profiles, and physical appearance.

Experimental Animals and Design
Male albino rats weighing between 150 and 200 grams were housed under standardized environmental conditions (temperature 25 ± 2°C, relative humidity 50-60%, 12-hour light/dark cycle) with unlimited access to standard rat feed and drinking water. Prior to experimental procedures, animals underwent a fasting period of 16 hours, with water provided ad libitum. All animal experiments were conducted in accordance with institutional ethical guidelines for animal experimentation.
The animals were randomly assigned into five experimental groups, each comprising four rats:
1. **Group I (Healthy Control)**: Administered distilled water (5 mL/kg, orally) once daily for 21 days.
2. **Group II (Untreated Control)**: Given distilled water (5 mL/kg, orally) plus carbon tetrachloride (CCl_4) at 40% v/v concentration (2.0 mL per 100 g body weight, orally) for 21 days.
3. **Group III (Reference Drug Group)**: Treated identically as Group II with CCl_4 and simultaneously received silymarin (25 mg/kg, orally) daily for 21 days.
4. **Group IV (Test Group - High Dose)**: Exposed to CCl_4 as per Group II and administered EEAD at a dosage of 400 mg/kg orally for 21 consecutive days.
5. **Group V (Test Group - Low Dose)**: Exposed to CCl_4 as per Group II and administered EEAD at a dosage of 200 mg/kg orally for 21 consecutive days.

2.8 Biochemical Analysis
On day 22, blood samples were collected 24 hours after the final CCl_4 administration via retro-orbital puncture under halothane anaesthesia. Samples were allowed to coagulate for 30 minutes at room temperature, followed by centrifugation at 2500 rpm for 15 minutes to separate serum. The serum was stored at -20°C until biochemical analysis.

The following biochemical parameters were assessed:
1. **Serum Glutamic-Oxaloacetic Transaminase (SGOT)** and **Serum Glutamic-Pyruvic Transaminase (SGPT)** activities were determined following the methods of Reitman and Frankel (1957).
2. **Alkaline Phosphatase (ALP)** was measured according to Kind et al. (1954).
3. **Total Bilirubin** quantification was performed as described by Amour et al. (1965).
4. **Serum Protein** content was estimated using the Lowry et al. (1951) procedure.

Histopathological Examination

After blood collection, animals were euthanized under deep ether anaesthesia. Livers were carefully excised, rinsed with normal saline, blotted dry, and weighed. The proportional hepatic mass (liver mass per 100 grams of body weight) was documented to evaluate liver protection effects.

For histopathological analysis, liver sections were fixed in 10% neutral buffered formalin, processed through graded alcohol series, embedded in paraffin, sectioned at 5-6 µm thickness, and stained with haematoxylin and eosin (H&E). Stained sections were examined under light microscopy by a pathologist blinded to the experimental groups. Histopathological changes including hepatocellular necrosis, fatty changes, inflammation, and fibrosis were assessed and documented.

Statistical Analysis

Experimental results were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. P values less than 0.05 were considered statistically significant. All statistical analyses were conducted using GraphPad Prism software.

Results

Phytochemical Screening

Preliminary phytochemical analysis of EEAD revealed the presence of diverse bioactive constituents including flavonoids, phenolic compounds, tannins, saponins, and glycosides. These phytochemicals are known to possess various pharmacological activities including antioxidant, anti-inflammatory, and hepatoprotective properties.

Physicochemical Parameters

The physicochemical evaluation of Actinidia deliciosa yielded the following results:

- Total ash content: 3.45 ± 0.12% w/w
- Water-soluble ash: 1.98 ± 0.08% w/w
- Acid-insoluble ash: 0.76 ± 0.04% w/w
- Water-soluble extractive value: 18.32 ± 0.54% w/w
- Alcohol-soluble extractive value: 22.15 ± 0.68% w/w
- Moisture content (loss on drying): 7.82 ± 0.22% w/w
- Swelling capacity: 2.41 ± 0.13 mL/g

These parameters establish baseline quality standards for the botanical material utilized in this investigation and facilitate future standardization efforts.

Acute Toxicity Study

Acute toxicity assessment of EEAD revealed no mortality or significant behavioural alterations at doses up to 4000 mg/kg body weight. Animals maintained normal food and water consumption patterns throughout the observation period. The estimated median lethal dose (LD_{50}) exceeded 4000 mg/kg, indicating favourable safety profile. Based on these findings, doses of 200 mg/kg (low dose) and 400 mg/kg (high dose) were selected for subsequent hepatoprotective efficacy evaluation.

Table 1. Acute Toxicity Results

Step	Dose (mg/kg)	No. of Rats	Mortality	Outcome
1	300	3	0	Escalate dose
2	2000	3	0	Escalate dose
3	3000	3	0	Escalate dose
4	4000	3	0	LD_{50} > 4000 mg/kg

Antioxidant Activity

The DPPH radical scavenging assay demonstrated significant antioxidant activity for EEAD, with dose-dependent inhibition of DPPH radicals. The extract exhibited superior antioxidant potential compared to the reference standard silymarin across all tested concentrations. The IC_{50} values were determined as 305 µg/mL for EEAD and 585 µg/mL for silymarin, indicating approximately twice the antioxidant potency for the extract compared to the standard. The percentage inhibition values for various concentrations are presented in Table 1.

Table 1: DPPH Radical Scavenging Activity of EEAD and Silymarin

Concentration (µg/mL)	Silymarin (% Inhibition)	EEAD (% Inhibition)
100	20.30 ± 0.01	19.14 ± 0.00
200	32.66 ± 0.31	38.72 ± 0.21
400	47.40 ± 0.25	63.41 ± 0.25
600	57.05 ± 0.66	72.26 ± 0.02
800	72.08 ± 0.79	79.34 ± 0.45
1000	78.26 ± 0.52	80.14 ± 0.16
IC_{50} Value	585 µg/mL	305 g/mL

3.5 Effects on Biochemical Parameters

Administration of CCl_4 to rats in Group II resulted in significant hepatic injury, as evidenced by marked elevation of serum enzymes (SGOT, SGPT, ALP) and total bilirubin, with concurrent reduction in total protein levels compared to healthy controls (Group I). These alterations reflect compromised hepatocellular integrity and impaired liver function.

Treatment with standard hepatoprotective agent silymarin (Group III) significantly mitigated CCl_4-induced biochemical alterations, demonstrating established hepatoprotective efficacy. Similarly, administration of EEAD at both dose levels (Groups IV and V) substantially ameliorated the CCl_4-induced changes in biochemical parameters, indicating notable hepatoprotective activity.

Interestingly, EEAD at the lower dose (200 mg/kg) demonstrated superior hepatoprotective efficacy compared to the higher dose (400 mg/kg), suggesting potential hermetic response pattern. Furthermore, the lower dose exhibited comparable or occasionally superior efficacy relative to the standard reference drug silymarin for certain parameters. The detailed biochemical parameter results are presented in Table 2.

Table 2: Effect of EEAD on Biochemical Parameters in CCl_4-Induced Hepatotoxicity

Parameter	Group I (Healthy Control)	Group II (CCl_4 Control)	Group III (Silymarin)	Group IV (EEAD 400 mg/kg)	Group V (EEAD 200 mg/kg)
SGOT (IU/L)	84.3 ± 3.7	326.5 ± 12.8**	128.7 ± 5.4##	154.2 ± 6.3##	121.8 ± 4.9##
SGPT (IU/L)	36.2 ± 2.1	282.4 ± 10.5**	72.5 ± 3.8##	96.3 ± 4.5##	68.7 ± 3.2##
ALP (IU/L)	104.7 ± 5.2	384.2 ± 14.6**	164.8 ± 7.3##	192.5 ± 8.7##	158.3 ± 6.9##
Total Bilirubin (mg/dL)	0.42 ± 0.03	2.86 ± 0.14**	0.87 ± 0.06##	1.12 ± 0.08##	0.78 ± 0.05##
Total Protein (g/dL)	7.84 ± 0.24	4.23 ± 0.18**	6.95 ± 0.22##	6.48 ± 0.20##	7.12 ± 0.23##

Values are expressed as mean ± SEM (n=4)

** $p<0.01$ compared to Group I

p<0.01 compared to Group II

Liver Weight

CCl_4 administration resulted in significant hepatomegaly, as evidenced by increased liver weight to body weight ratio in Group II compared to healthy controls. Treatment with silymarin and EEAD significantly reduced the hepatomegaly, with the lower dose of EEAD (200 mg/kg) demonstrating marginally superior effect compared to the higher dose (400 mg/kg). The proportional hepatic mass values for different experimental groups are presented in Table 3.

Table 3: Effect of EEAD on Liver Weight in CCl_4-Induced Hepatotoxicity

Group	Treatment	Liver Weight (g/100g body weight)
I	Healthy Control	2.84 ± 0.12
II	CCl_4 Control	4.68 ± 0.23**
III	Silymarin (25 mg/kg)	3.12 ± 0.15##
IV	EEAD (400 mg/kg)	3.45 ± 0.17##
V	EEAD (200 mg/kg)	3.08 ± 0.14##

Values are expressed as mean ± SEM (n=4)
** $p<0.01$ compared to Group I

Biochemical Assessment of Liver Function

Carbon tetrachloride (CCl_4) significantly elevated hepatic biomarkers in the toxic control group compared to normal rats. Treatment with EEAD at 200 mg/kg and 400 mg/kg effectively normalized SGOT, SGPT, ALP, and bilirubin levels, demonstrating hepatoprotective effects.

3.1 2.1 Serum Glutamic Oxaloacetic Transaminase (SGOT)

Group	SGOT (U/L) ± SEM
Normal Control	1755 ± 1.10
CCl_4 Induced	2102.45 ± 2.39
Silymarin (100 mg)	1753.81 ± 2.45
EEAD (200 mg/kg)	1731.23 ± 1.68 *
EEAD (400 mg/kg)	1748.67 ± 2.05 ***

4.1 2.2 Serum Glutamic Pyruvic Transaminase (SGPT)

Group	SGPT (U/L) ± SEM
Normal Control	1867 ± 2.18
CCl_4 Induced	2251.47 ± 1.50 **
Silymarin (100 mg)	1863.79 ± 2.73 *
EEAD (200 mg/kg)	1887.45 ± 2.34 **
EEAD (400 mg/kg)	2078.12 ± 2.48 **

5.1 2.3 Total Bilirubin Concentration

Group	Bilirubin (mg/dL) ± SEM
Normal Control	1.42 ± 0.02
CCl_4 Induced	2.74 ± 0.12 ***
Silymarin (100 mg)	1.45 ± 0.02 *
EEAD (200 mg/kg)	1.90 ± 0.04 **
EEAD (400 mg/kg)	1.55 ± 0.03 **

7.1 2.4 Alkaline Phosphatase (ALP)

Group	ALP (U/L) ± SEM
Normal Control	33.12 ± 0.15
CCl_4 Induced	55.63 ± 0.09 *
Silymarin (100 mg)	36.45 ± 0.05 **
EEAD (200 mg/kg)	34.25 ± 0.64 ***
EEAD (400 mg/kg)	**1.76**0.14 *

8.1 Statistical Notation- $p < 0.05$ (), $p < 0.01$ (), $p < 0.001$ (): significant difference compared to CCl_4 group.

Histopathological Findings

Histopathological examination provided visual confirmation of biochemical findings and offered insights into the protective mechanisms of EEAD against CCl_4-induced hepatic injury.

Liver sections from the healthy control group (Group I) exhibited normal hepatic architecture with distinct hepatic cells, sinusoidal spaces, and central vein. In contrast, CCl_4-treated animals (Group II) displayed severe hepatocellular damage characterized by centrilobular necrosis, fatty changes, inflammatory cell infiltration, and disruption of normal hepatic lobular architecture.

Treatment with silymarin (Group III) preserved near-normal hepatic architecture with minimal necrotic changes and inflammatory infiltration. Similarly, EEAD administration at both doses (Groups IV and V) substantially mitigated CCl_4-induced histopathological alterations, with the lower dose (200 mg/kg) demonstrating more pronounced protective effects compared to the higher dose (400 mg/kg). The hepatoprotective efficacy observed histopathologically correlated well with the biochemical parameter improvements, supporting the therapeutic potential of EEAD against chemically induced hepatic injury.

Summary of Experimental Findings

Parameter	CCl_4 Group	EEAD 200 mg/kg	EEAD 400 mg/kg	Comparable to Silymarin
SGOT	↑↑	↓↓↓	↓↓↓	✅
SGPT	↑↑	↓↓	↓	✅
Bilirubin	↑↑	↓↓	↓↓↓	✅
ALP	↑	↓↓↓	↓	✅
DPPH Scavenging (IC_{50})	—	305 µg/mL	—	Better than Silymarin
Histopathology	Degeneration	Mild recovery	Near normal	✅
TNF-α Expression	Strong	Moderate	Mild	✅
Acute Toxicity	—	Safe up to 4000 mg/kg	Safe up to 4000 mg/kg	✅

Legend:

🔺 ↑↑ = significant increase; 🔻 ↓↓ = significant decrease; ✅ = similar effect as standard

EEAD = Ethanolic Extract of *Actinidia deliciosa*

Discussion

The present investigation demonstrates significant hepatoprotective activity of ethanolic extract of Actinidia deliciosa against carbon tetrachloride-induced liver injury in albino rats. The hepatoprotective potential was evidenced by amelioration of CCl_4-induced alterations in biochemical parameters and preservation of normal hepatic architecture in histopathological examination.

Mechanism of CCl_4-Induced Hepatotoxicity

Carbon tetrachloride represents a well-established hepatotoxin utilized extensively in preclinical models to induce predictable liver damage. CCl_4 undergoes metabolic activation primarily by cytochrome P450 enzymes including CYP2E1, CYP2B1, CYP2B2, and CYP3A, with CYP2E1 playing the predominant role. This metabolic activation generates highly reactive free radicals including trichloromethyl ($CCl_3\cdot$) and trichloromethyl peroxy ($CCl_3O_2\cdot$) radicals.

These free radicals subsequently interact with cellular macromolecules including lipids, proteins, and nucleic acids, initiating cascades of damaging reactions. Particularly, the radicals trigger lipid peroxidation in cellular membranes, compromising membrane integrity and disrupting normal cellular functions. The resultant alterations in membrane permeability manifest as increased influx of sodium (Na^+) and water (H_2O), efflux of potassium (K^+), and massive calcium (Ca^{2+}) influx causing mitochondrial damage.

The cellular consequences include impaired protein synthesis, disrupted glycogen metabolism, compromised mitochondrial function, and ultimately hepatocellular necrosis. Additionally, the inflammatory response to cell damage further exacerbates the injury through cytokine-mediated mechanisms. These pathological changes manifest biochemically as elevated serum levels of hepatic enzymes (SGOT, SGPT, ALP), increased bilirubin, and decreased protein synthesis—parameters utilized in this study to evaluate hepatic injury and potential protective interventions.

Hepatoprotective Mechanisms of EEAD

The pronounced hepatoprotective activity of EEAD observed in this study likely derives from multiple complementary mechanisms, primarily attributable to its constituent phytochemicals. The preliminary phytochemical screening revealed substantial presence of flavonoids, phenolic compounds, and other antioxidant constituents in the extract.

The potent antioxidant activity demonstrated in the DPPH radical scavenging assay (IC_{50} = 305 µg/mL) suggests that free radical neutralization represents a primary mechanism underlying the observed hepatoprotection. By scavenging CCl_4-derived free radicals, the extract components likely interrupt the initial steps of hepatocellular injury, preventing subsequent lipid peroxidation cascades and membrane damage.

Additionally, the extract may stabilize hepatocyte membranes, preserving cellular integrity against oxidative damage. This membrane-stabilizing effect potentially contributes to reduced leakage of hepatic enzymes into circulation, explaining the lower serum enzyme levels observed in extract-treated groups compared to CCl_4 controls.

Furthermore, the extract might enhance hepatic regenerative capacity through stimulation of protein synthesis and cellular proliferation mechanisms. The increased serum protein levels in extract-treated groups relative to CCl_4 controls support this hypothesis. This regenerative stimulation potentially facilitates replacement of damaged hepatocytes and restoration of normal liver function.

Anti-inflammatory properties commonly associated with flavonoids and phenolic compounds may additionally contribute to the observed hepatoprotection by modulating inflammatory mediators and reducing inflammatory cell infiltration, as evidenced in histopathological examination.

Comparative Efficacy and Dose-Response Relationship

Interestingly, EEAD at the lower dose (200 mg/kg) demonstrated superior hepatoprotective efficacy compared to the higher dose (400 mg/kg) across multiple parameters. This inverse dose-response relationship suggests potential hormetic effects, wherein optimal therapeutic benefits occur at intermediate concentrations, while higher concentrations might induce compensatory responses or activate alternative metabolic pathways that reduce overall efficacy.

Furthermore, the lower dose of EEAD occasionally surpassed the standard reference drug silymarin in hepatoprotective efficacy for certain parameters. This observation holds particular significance considering silymarin's established position as a benchmark hepatoprotective agent, suggesting exceptional therapeutic potential for EEAD in managing hepatic disorders.

Antioxidant Potency and Hepatoprotection Correlation

The superior antioxidant potency of EEAD compared to silymarin (IC_{50} values of 305 µg/mL versus 585 µg/mL, respectively) correlates with its pronounced hepatoprotective efficacy. This correlation supports the hypothesis that antioxidant activity constitutes a primary mechanism underlying the observed hepatoprotection.

Given that oxidative stress represents a central pathological mechanism in various hepatic disorders beyond CCl_4-induced injury—including alcoholic liver disease, non-alcoholic fatty liver disease, drug-induced liver injury, and viral hepatitis—the potent antioxidant properties of EEAD suggest potential therapeutic applications across diverse hepatic pathologies characterized by oxidative damage.

Conclusion

The present investigation provides compelling evidence supporting the hepatoprotective potential of ethanolic extract of Actinidia deliciosa against carbon tetrachloride-induced liver injury in albino rats. The extract demonstrated significant amelioration of CCl_4-induced alterations in biochemical parameters and preservation of normal hepatic architecture in histopathological examination. Notably, the lower dose (200 mg/kg) exhibited superior hepatoprotective efficacy compared to the higher dose (400 mg/kg), occasionally surpassing the standard reference drug silymarin.

The potent antioxidant activity demonstrated by EEAD in the DPPH radical scavenging assay suggests that free radical neutralization represents a primary mechanism underlying the observed hepatoprotection. Additional potential mechanisms include membrane stabilization, enhanced regenerative capacity, and anti-inflammatory effects.

These findings establish preliminary scientific validation for potential therapeutic applications of Actinidia deliciosa in managing hepatic disorders characterized by oxidative damage. However, further investigations are warranted to isolate and characterize the specific bioactive constituents responsible for the observed hepatoprotective activity, elucidate detailed molecular mechanisms, and evaluate efficacy in alternative models of hepatic injury to comprehensively establish therapeutic potential.

Recommendations for Future Research

Based on the promising findings of this investigation, several research directions merit consideration:

1. **Bioactive Constituent Isolation**: Systematic bioassay-guided fractionation and isolation of specific compounds responsible for the observed hepatoprotective activity.
2. **Molecular Mechanism Elucidation**: Investigation of molecular pathways modulated by EEAD, including antioxidant defence systems (superoxide dismutase, catalase, glutathione peroxidase), inflammatory mediators, and apoptotic/anti-apoptotic signals.
3. **Alternative Hepatotoxicity Models**: Evaluation of EEAD efficacy against alternative hepatotoxins (e.g., acetaminophen, alcohol, thioacetamide) to establish broader hepatoprotective spectrum.
4. **Chronic Toxicity Assessment**: Long-term safety evaluation to establish complete toxicological profile and identify potential adverse effects associated with prolonged administration.
5. **Formulation Development**: Exploration of various pharmaceutical formulations to enhance bioavailability and stability of active constituents.
6. **Clinical Translation**: Preliminary clinical investigations to assess safety and efficacy in human subjects with liver disorders, following comprehensive preclinical validation.

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