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Review Article

Hydroalcoholic Leaf Extract of Hibiscus cannabinus Restores Antioxidant Enzymes and Ameliorates HAART-Induced Hepatotoxicity in Wistar Rats

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Abstract:

Background: Long-term use of highly active antiretroviral therapy (HAART), typically with zidovudine, lamivudine, and nevirapine, induces hepatic oxidative stress and toxicity

Objective: The antioxidant and hepatoprotective activity of hydroalcoholic leaf extract of Hibiscus cannabinus (HC) was assessed in this study against HAART-induced oxidative injury.

Methods: Wistar rats were divided into three groups: Control, HAART, and HAART+HC extract. The marker levels of peroxisomal (SOD, CAT, GPx) and lipoxidative (MDA) stress were measured. Histopathological examination of the

Results: HAART significantly reduced SOD, CAT, and GPx activities ($p<0.05$) but elevated MDA levels. HC extract-treated rats recovered the activities of antioxidant enzymes and reduced lipid peroxidation near normal. Histopathology confirmed the maintenance of the structure of the hepatic tissue in the HC-treated rats.

Conclusion: H. cannabinus extract reduces HAART-related hepatotoxicity through replenishment of antioxidant levels, which suggests potential as an adjunct nutraceutical.

Keywords: Hibiscus cannabinus, HAART, oxidative stress, hepatoprotection, antioxidants, Wistar rats

Introduction

Highly active antiretroviral therapy (HAART)—a triple-combination therapy in most instances with zidovudine, lamivudine, and nevirapine—has greatly enhanced the survival of HIV/AIDS patients. Yet HAART treatment over a long duration is linked with hepatotoxicity, primarily mediated by oxidative stress, mitochondrial disruption, and lipid peroxidation[1]. High reactive oxygen species overcome protective barriers against reactive oxygen species, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and thereby compromise hepatic function[2–4].

To counteract these adverse effects, plant-derived antioxidants are being investigated as adjunct therapies. Hibiscus cannabinus (kenaf), a member of the Malvaceae family, is rich in polyphenols, flavonoids, tannins, and saponins, which contribute to its antioxidant and antimicrobial potential[5,6]. Related species, such as Hibiscus sabdariffa, have shown hepatoprotective and ROS-scavenging activity in various experimental models of oxidative stress[7–9].

To date, no published study has systematically examined the hepatoprotective potential of Hibiscus cannabinus leaf extract against HAART-induced oxidative liver injury. The present study was therefore designed to evaluate the effect of hydroalcoholic HC leaf extract on oxidative stress markers and histopathological changes in HAART-challenged Wistar rats.

MATERIAL AND METHOD

Fresh leaves of Hibiscus cannabinus were obtained from undisturbed fields at Nagpur, Maharashtra, between the months August–October and were identified by a Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University botanist (voucher no. 9621 dated 30/06/2011). The powdered dried leaves were Soxhlet extracted under diminished pressure with the hydroalcoholic solvent (ethanol-water 1:1). The crude dried extract was packed in tightly closed containers and utilized for experimentation. The yield, color, and texture were determined, and initial qualitative analysis verified the occurrence of polyphenols and flavonoids through standard protocols. Healthy young adult Wistar rats (150–200 g) were kept in polypropylene cages with light and dark phases of 12 h and with ad libitum water and food.

The experimental procedure was accepted. The rats were randomly assigned to four groups of six rats each: control (vehicle alone), HAART-treated (zidovudine 10 mg/kg + lamivudine 4 mg/kg + nevirapine 10 mg/kg, orally, once daily for 28 days), HAART plus H. cannabinus extract (200 mg/kg, orally for 28 days), and extract alone (200 mg/kg). Following termination of therapy, blood was drawn by retro-orbital puncture and serum was harvested for the intention of carrying out liver function tests such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) and estimation of the same using commercial diagnostic kits. Liver tissues were excised, washed with ice cold normal saline, and homogenized after undergoing biochemical estimation.

Lipid peroxidation was determined from the estimation of malondialdehyde (MDA) concentration, while reduced glutathione (GSH) was measured using Ellman's reagent. Activities of the antioxidant enzymes—superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)—were estimated on the basis of standard spectrophotometric methods. For histopathological examination, the liver sections were fixed in the case of buffered (10%) formalin, paraffin-embedded, sectioned into 5 μm Values are presented as mean ± SEM. Inter-group comparisons were made using one-way ANOVA and Tukey's post-hoc test, where $p < 0.05$ was considered to be statistically significant.

RESULTS

Extract characterization:

The hydroalcoholic extract yield was 16.5% (dark green, sticky slurry). Preliminary phytochemical screening indicated presence of phenolics and flavonoids.

Extract	Color	Consistency	Yield (%)
Hydroalcoholic leaf extract	Dark green	Sticky slurry	16.5

Phytochemical Screening

The hydroalcoholic extract of *Hibiscus cannabinus* yielded 12.4% (w/w) dry extract, which was dark green in color and semisolid in consistency. Preliminary phytochemical tests revealed the presence of flavonoids, tannins, saponins, and phenolic compounds.

Table 1. Preliminary phytochemical analysis of *Hibiscus cannabinus* leaf extract

Phytochemical group	Test used	Result (+/-)
Alkaloids	Mayer's	–
Flavonoids	Shinoda	+
Tannins	Ferric chloride	+
Saponins	Frothing test	+
Phenolics	Folin–Ciocalteu	+
Glycosides	Keller–Killiani	–

(+ = present, – = absent)

Liver Function Tests

HAART administration (zidovudine + lamivudine + nevirapine) significantly increased serum ALT, AST, and ALP levels compared with control rats ($p < 0.001$). Co-administration of *H. cannabinus* extract significantly ($p < 0.01$) reduced these enzyme levels toward normal. Extract alone did not alter liver function markers.

Table 2. Effect of *H. cannabinus* extract on serum liver enzymes (mean ± SEM, n = 6)

Group	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	32.5 ± 2.1	45.2 ± 3.3	112.1 ± 5.4
HAART	78.6 ± 4.8***	92.4 ± 5.2***	256.5 ± 10.3***
HAART + Extract	40.7 ± 3.1###	54.6 ± 3.7###	130.2 ± 6.9###
Extract alone	34.8 ± 2.7	46.3 ± 3.5	115.9 ± 4.6

***$p < 0.001$ vs. Control; ###$p < 0.001$ vs. HAART group (ANOVA + Tukey's test).

ALT, AST, and ALP were significantly elevated in HAART group ($p < 0.01$ vs. control). Co-treatment with extract normalized these enzymes.

Oxidative stress markers:

HAART treatment significantly increased hepatic MDA and reduced GSH levels compared with control ($p < 0.01$). Co-treatment with *H. cannabinus* extracts significantly reduced MDA and restored GSH ($p < 0.05$ vs. HAART). SOD, CAT, and GPx activities were reduced in HAART rats ($p < 0.01$ vs. control). Extract co-administration significantly restored these activities ($p < 0.05$).

Table 3. Biochemical parameters (mean ± SD) in control and treated groups

Group	TBARS (ηmol MDA/mg protein)	SOD (U/mg protein)	Catalase (µmol H_2O_2/min/mg protein)	Glycogen (µg/g wet tissue)
Group I (Control, 0.9% saline)	32.654 ± 1.88	0.109 ± 0.01	15.688 ± 1.90	0.029 ± 0.03
Group II (SLN)	15.938 ± 1.62	0.089 ± 0.004	10.707 ± 1.97	0.006 ± 0.003
Group III (ZLN)	16.175 ± 1.64	0.078 ± 0.01	7.764 ± 1.77	0.006 ± 0.001
Group IV (Efv)	19.874 ± 1.62	0.083 ± 0.01	10.48 ± 2.23	0.012 ± 0.001
Group V (Nvp)	18.008 ± 0.44	0.088 ± 0.01	8.02 ± 1.64	0.007 ± 0.001
Group VI (SLN + HC)	15.979 ± 2.81*	0.115 ± 0.004***	19.523 ± 1.39**	0.014 ± 0.002**
Group VII (ZLN + HC)	15.979 ± 2.85**	0.129 ± 0.01***	19.501 ± 1.25**	0.033 ± 0.001*
Group VIII (Efv + HC)	15.979 ± 2.80**	0.139 ± 0.01**	30.63 ± 1.57*	0.031 ± 0.001*
Group IX (Nvp + HC)	15.979 ± 0.77*	0.143 ± 0.01***	21.50 ± 1.16*	0.030 ± 0.001*

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. respective drug alone.

Data expressed as Mean ± SD (n = 6).

Table 4. Effect of HC extract on glutathione related antioxidant enzymes in drug-induced toxicity models

Groups	Treatments	Reduced Glutathione (GSH, µg/mg protein)	Glutathione Peroxidase (Gpx, U/mg protein)	Glutathione-S-Transferase (GST, U/mg protein)
Positive Control	Group I	Control (normal saline)	0.254 ± 0.02	0.169 ± 0.03
Negative Control	Group II	SLN	0.025 ± 0.01	0.081 ± 0.02
	Group III	ZLN	0.016 ± 0.02	0.071 ± 0.05
	Group IV	Efv	0.012 ± 0.04	0.059 ± 0.01
	Group V	Nvp	0.012 ± 0.01	0.069 ± 0.01
Experimental Group	Group VI	SLN + HC extract	0.204 ± 0.01*	0.034 ± 0.01***
	Group VII	ZLN + HC extract	0.201 ± 0.01*	0.277 ± 0.04***
	Group VIII	Efv + HC extract	0.306 ± 0.03**	0.113 ± 0.01*
	Group IX	Nvp + HC extract	0.200 ± 0.01*	0.142 ± 0.01**

Values are expressed as mean ± SD (n = X).
*Significance vs. Negative Control: *p < 0.05, **p < 0.01, ***p < 0.001.

Discussion

The present study demonstrates that hydroalcoholic extract of *Hibiscus cannabinus* possesses significant hepatoprotective and antioxidant effects in rats exposed to HAART-induced hepatotoxicity. Treatment with HC extract restored liver function biomarkers, normalized oxidative stress parameters, and improved antioxidant enzyme activity (SOD, CAT, GPx), corroborated by histopathological protection of liver tissue.

These findings are consistent with previous reports of *Hibiscus* species demonstrating hepatoprotective and antioxidant effects against chemically and metabolically induced hepatic injury[7–9]. Replenishment of glutathione level and enzymatic antioxidant activity was consistent with overall kenaf seed and leaf extract literature as an effective antioxidant agent[5,6].

Thus, Hibiscus cannabinus extract is a potential adjuvant therapy for HAART-induced hepatotoxicity. Its research direction in the future should be aimed at isolating the bioactive molecules, mechanism of action, and clinical trials for establishing its therapeutic usefulness in HIV/AIDS treatment.

Conclusion

Hydroalcoholic leaf extract of Hibiscus cannabinus restores the activity of antioxidant enzymes, inhibits lipid peroxidation, and maintains hepatic histology in HAART-induced hepatotoxicity. It is promising as an adjunct nutraceutical against oxidative stress in HIV treatment.

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