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Research Article

Hepato-restorative Activity of Methanolic Extracts of *Coccinia grandis* L. Voigt. in CCl_4 - Intoxicated Rats

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ABSTRACT

Background: *Coccinia grandis* has pharmacological activities such as antioxidant, anti-ulcer, anti-inflammatory, anti-hypersensitive, anti-hyperglycaemic, anti-cancer and hepatoprotective. **Objective:** This work is aimed to investigate an alternative low-cost green drug with hepatoprotective potential from methanolic extract of the leaf, stem and their corresponding calli of *Coccinia grandis*. **Materials and Methods:** Using 42 Albino Wistar rats divided into seven groups each group containing 6 rats. 1.5ml/kg bw of CCl_4 diluted in olive oil was orally injected for fourteen days and methanolic extracts of parent plant parts, callus and silymarin, and on the last day of treatment, experimental rats were anesthetized, blood and organ removed for the biochemical and histopathological analysis. **Results:** This work is aimed to investigate an alternative low-cost green drug with hepatoprotective potential. Liver damage was induced by CCl_4 (1.5 ml/kg body weight) in Wistar albino rats and recovery was noted by treating with Silymarin (100mg/kg bw), a known standard herbal drug and by treating with crude methanolic extract of leaf and stem parts of *Coccinia grandis* and their corresponding calli (leaf callus and stem callus at 180mg/kg bw) in terms of marked decrease in CCl_4- increased SGOT (Serum glutamic oxaloacetic transaminase), SGPT (Serum glutamic pyruvic transaminase), ALP (Alkaline phosphatase), TB (Total bilirubin) and rise in TP (Total protein) compared to untreated control group. Histopathological studies of hepatocytes provide evidence of the centrilobular vacuolar degeneration and recovery by Silymarin or treatment with plant and callus extracts. **Conclusion:** Biochemical and histopathological examination proved the hepatoprotective potential of calli and parent plant parts (leaf, stem) of *Coccinia grandis*.

Keywords: *Coccinia grandis*, callus, CCl_4, Liver, GC-MS, Silymarin.

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INTRODUCTION

The liver is a major detoxifying organ of our body, and it performs various of metabolic activities[25]. Today numerous drugs and toxic substances cause liver damage, increase mortality and morbidity rates in the human population, due to a lack of proper diagnosis and management. No therapy has so far been concretely developed to prevent the growth of cirrhosis and fibrosis[4,23], while more than 75% of cases of liver transplantation or death are due to idiosyncratic medication[15]. In most of the cases damage to the liver is caused by the ingestion of chemical substances leading to fibrosis and cirrhosis resulting finally into liver failure. For experimental purposes, CCl_4 (carbon tetrachloride) is commonly used for inducing liver injury[16] through cytochrome P_{450} which causes metabolic activation of CCl_4 and produces trichloromethyl (CCl_3^*) and peroxy trichloromethyl ($OOCCl_3^*$) leading to initiation of lipid peroxidation and liver damage. A single dose of CCl_4 causes oxidative stress leading to increased steatosis and centrilobular necrosis in the liver. The endoplasmic reticulum (ER), a major cytoplasmic functional organelle, is responsible for the production of protein, maturation, modification and the homeostasis of intracellular calcium. The unfolded protein and imbalance of calcium homeostasis leads to ER stress[6]. Under stress, ER triggers three ER membrane proteins responsible for the induction of adaptive immune response such as activating transcription factor (ATF6), serine/threonine kinase PKR-like ER kinase (PERK) and inositol-requiring enzyme-1(IRE1). ER stress also causes many disorders such as neurodegeneration, obesity, diabetes, and inflammation[8]. Inflammation is the main indication factor to determine illness and death in nearly all diseases. In the initial stage of inflammation, interferon (INF), interleukin (IL) IL-6, IL-12 and IL18 are upregulated and promoted to macrophage and neutrophils leading to the production of inflammatory mediators, including Prostaglandin (PGE2) and Nitric oxide (NO). ROS activates during the inflammation condition, enhancing or reducing the inflammatory response, initiating lipid peroxidation of the membrane, secreting toxic macromolecules and upregulating NF-kB[5]. Lipid peroxidation, reduced antioxidant activities and formation of free radicals are symptoms of liver damage during the treatment of CCl_4. Cytochrome P_{450} known as terminal hepatic mixed function oxidase enzyme catalyzes dehalogenation of carbon tetrachloride. During the treatment of CCl_4, leakage of lipid and unsaturated fatty acids starts leading to intracellular and cellular membrane damage. All the broken products change into predominantly reactive aldehydes causing further damage to liver cells. To protect from damage, specific antioxidants have been identified such as Silymarin, obtained from *Silybum marianum*, as a potent antioxidant drug to protect the liver from injury[3]. Medicinal plants are sources of the richest cache of drugs and bioactive compounds, used in the traditional system of medicine, modern medicine, folk medicine, food supplements, pharmaceuticals, synthetic drugs, and chemical entities[20]. In this light, other potential plants reported in ethnobotanical literature were also

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assessed. *Coccinia grandis* L. Voigt., commonly known as "Ivy gourd", belonging to the family Cucurbitaceae and native to North Central East Africa[7], is known for its therapeutic properties such as antioxidant, anti-fungal, anti-ulcer, analgesic, anti-inflammatory (for wound healing), anti-hypersensitive, anti-hyperglycaemic, anti-microbial, anti-cancer and antipyretic, hepatoprotective (against jaundice and hepatitis), etc.[13,11]. The present work has been aimed at phytochemical profiling of *C. grandis* for assessment of its hepatoprotective potential.

MATERIALS AND METHODS

Material collection

Leaves and stem of *Coccinia grandis* (L.) Voigt. were collected in September 2022 from the Department of Botany, Chaudhary Charan Singh University Meerut, Uttar Pradesh and calli from the respective plant parts were raised on MS media supplemented with 1.0mg/L NAA + 0.5 mg/L kn and 1.0 2,4-D mg/L +0.5 kn mg/L[21] Figure 1 a, b and c. The study material was identified and verified by the Botanical Survey of India, Central National Herbarium Howrah (BSI, Howrah Calcutta, India), and deposited as brochure no. CNH/Tech. II/2022/63, BSI India.

Preparation of methanolic extracts from parent plant parts and calli

For the preparation of extracts, leaves, stem and calli (leaves and stem) of *C. grandis*, were dried in the oven at 50°C temperature, and pulverized by an electric grinder to a fine powder.

1gm fine powder of plant and callus was added to the thimble and run in 95% methanolic solution at 60 °C temperature using Soxhlet for 48 hours. The extracted supernatant was filtered using Whatman no. 1.0 filter paper and lyophilized. The lyophilized fine powder was dissolved in sterilized distilled water at1.0mg/ml concentration for further analysis [26].

Drugs and chemicals used

The following chemicals and drugs were used in this work

Silymarin (Sigma-Aldrich, USA) and carbon tetrachloride (CCl_4, Sigma-Aldrich, USA) were gifted from PBRI Bhopal, standard kits for assay of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin (TB) and total protein (TP) were purchased from Span diagnostic Ltd. Bhopal, Madhya Pradesh.

Experimental models (Animals)

Forty-two Male albino Wistar rats (200±20g-BW) were used in this study, acquired from PBRI Bhopal Madhya Pradesh. All the experimental models (rats) were housed in polypropylene cages in a well-ventilated room and maintained at 25±2°C temperature, under 12 hours light/dark cycle in a pathogen-free environment. According to OECD's guidelines, food was provided ad libitum as pellets and water for the experimental models (rats). All the experimental study was started after seeking consent from the Institutional Animal Ethical Committee (IAEC). The experimental work was started after acclimatization (12 days) of rats and divided into seven groups of six rats each. The dosages of rats (each group) were determined according to OECD guidelines (2000)[14].

Acute toxicity evaluation

The acute toxicity of the extracts was evaluated according to the OECD guidelines (2000). Albino Wistar rats (n=6) were used for the evaluation of acute toxicity. The plant and callus extracts were prepared in methanol (95%). The methanolic extract of *C. grandis* and callus was administered at single dose of 180mg/kg. The dose of methanolic extracts of *C. grandis* leaf, stem and callus was selected based on the previous potential studies[24-12-3]. Silymarin (100mg/kg) was administered to the experimental models (Albino Wistar rats) orally[24].

Experimental design

The hepatoprotective potential of methanolic extracts of parent plant parts (stem and leaf) and callus (leaf callus and stem callus) were analyzed using 42 Albino Wistar rats divided into seven groups each group containing 6 rats. 1.5ml/kg bw of CCl_4 diluted in olive oil (CCl_4: Olive oil;1:1) was orally injected[19] for fourteen days and methanolic extracts of parent plant parts, callus and silymarin (Classical standard drug which inhibits the formation of lipid peroxide and stabilizes liver cell membrane. It is also considered to be effective in protecting liver from fibrosis), were administered through intraperitoneal injection to assess restoration from CCl_4 induced hepatic injury[1].

Dose design

Group I (Normal control): No treatment (Saline water)

Group II (CCl_4):1.5ml/kg-bw/day (1.5ml/kg bw in olive oil)

Group III (CCl_4 +Silymarin): 100mg/kg bw/day

Group IV (CCl_4 + CL):180mg/kg bw/day

Group V (CCl_4 + CS): 180mg/kg bw/day

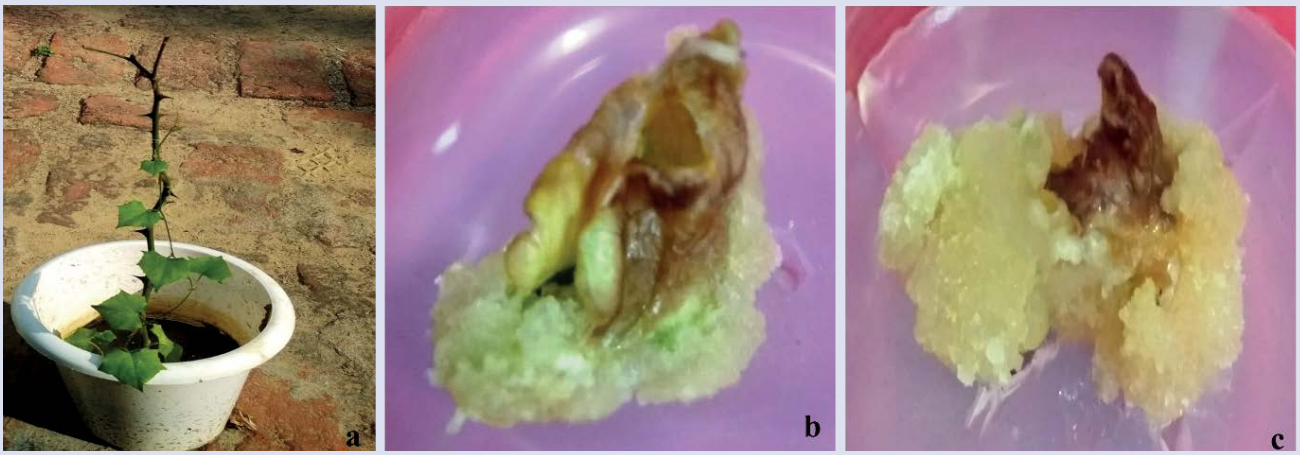

Figure 1: a. mother plant; b. leaf callus and c. stem callus.

Group VI (CCl_4 + CL-C): 180mg/kg bw/day

Group VII (CCl_4 + CS-C): 180mg/kg bw/day

Upon dosage completion, the models (Albino Wister rats) were subjected to dissection and animals were euthanized by decapitation after 24 hours of CCl_4 administration. The liver was removed immediately after anesthetization and washed in cold saline water at 4°C followed by blotting it dry. The washed and isolated portion of the liver was used for histopathological studies and blood samples were obtained by retro-orbital puncture and then centrifuged at 3000 rpm for 10 min. at 4°C to isolate the serum. The obtained serum was preserved at -20°C for further biochemical analysis.

Biochemical analysis

The biochemical or serum markers such as Serum glutamic oxaloacetic transaminase (SGOT)[18], Serum glutamic pyruvic transaminase (SGPT)[9], Alkaline phosphatase (ALP)[27], Total bilirubin (TB)[28] and Total protein (TP) were estimated through liver function assays utilizing the standard manual of Span diagnostic kit.

Statistical analysis

The obtained results were statistically (Mean± SD) analyzed through one-way analysis of variance (ANOVA) and multiple comparison procedures available in the statistical package program version (SPSS 25.0). The significance level is expressed as $P<0.001$ for the Bonferroni test.

Histopathological studies

After the anesthetization of the experimental rats, the blood samples were collected through the retro-orbital puncture and the liver was removed immediately and submerged in 10% formalin solution for histopathological evaluation. The obtained tissues were processed by dehydration using increasing concentration grades of alcohol followed by clearing with toluene and infiltration with molten paraffin wax grades for a specific time duration. The processed tissues were fixed in fresh paraffin wax and waited until the wax was set. The tissues were then sectioned and stained by eosin for structural identification of cells. Stained tissues were again processed in different grades of alcohol, cleared by xylene, and fixed in Canada balsam. Finally, the prepared slides were viewed under the microscope using a 10x objective and 10x eyepiece lens for histopathological changes.

Gas chromatography-Mass spectrophotometry Profiling (GC-MS)

GC-MS profiling of methanolic extracts of leaf, stem and calli was carried out using GC-MS-QP2010 ultra equipment (SHIMADZU, Kyoto, Japan) at Jawaharlal Nehru University, Delhi (Science Instrumentation Centre, AIRF). GC-MS-QP2010 ultra equipment with a capillary column (RT×5MS) with 30mm×0.25mm (length ×diameter) and 0.25µm film thickness and auto-injector (AOC-20i) and headspace sampler (AOC-20s), Ion source was set at 230°C temperature and interface at 270°C temperature with the mass selective detector used and injector temperature reconciled to 260°C. The initial temperature of 50°C was applied for 3 min, raised 10 °C/min up to 260 °C at a ramp rate of 15°C/min. Pure helium gas (99.99%) was used as a carrier gas with 40.9 cm/sec linear velocity at 90.5 kPa and a total flow of 16.3 ml/min, with 1.21 ml/min column flow. The obtained phytocompounds present in methanolic extracts were identified based on a comparison of their peak area %, retention time (min), mass spectral height and peak height with spectral database of the National Institute of Standards and Technology (NIST, US) and WILEY 8 libraries[21].

RESULTS

GC-MS profiling

GC-MS profiling of methanolic fraction of CL, CS, CL-C and CS-C of *Coccinia grandis* revealed 86 phytocompounds that may have a useful role in the hepatoprotective activity. Selected data of molecular weight (Mw), molecular formula (MF), retention time (RT) and area % are presented in Table 1 (extracted with permission from Singh et al. 2023)[21]. 10-Nonadecanol (CL; 33.42%), 9-Octadecenamide (CL-C; 57.87%, CS;85%, CS-C; 64.36%) showed maximum area % in GC-MS profiling. Beta-Amyrin; 1-Chloroheptacosane; Neophytadiene; 1-Octadecanol; Canadine; Protopine; Isopropyl linoleate; Behenic alcohol; Olean-12-en-3-ol, acetate, (3-beta)-; Benzene-propanoic acid,3,5-bis(91,1-dimethylethyl)-4-hydro; 1-Eicosanol; Gamma-sitosterol; Heneicosane and Hexadecenoic acid-methyl ester, 5-(Hydroxymethyl)-2- (dimethoxy methyl) furan was also recorded as potent phytocompounds with possible hepatoprotectives activity[21].

The effect of methanolic extracts of leaf, stem of *Coccinia grandis* and their corresponding calli on biological markers:

The level of hepatic markers in the serum of all the groups is presented in Figure 2. The biochemical markers used were SGOT, SGPT, ALP, TB and TP. The first four of the mentioned markers are known to represent hepatic injury, membrane breakdown and leakage of bilirubin breaking the red blood cells, whereas the fifth marker relates to a decline in protein metabolism with a decline in TP, hence the liver injury is evidenced by lowering of TP against standard values or controls.

The results showed a significant elevation in the levels of SGOT, SGPT, ALP and TB after CCl_4 treatment over the control group. Treatment with silymarin (100 mg/kg bw) on CCl_4-treated rats, decreased the levels of SGOT, SGPT, ALP and TB indicating a reduction in degradation and transportation of metabolites across the hepatocyte membrane, while increasing the level of TP in serum indicating active protein metabolism improving digestive liver function comparable with the control group. The single doses (180 mg/kg bw) of CL, CS, CL-C and CS-C significantly (**, $p< 0.001$) lowered the levels of SGOT, SGPT, ALP and TB and insignificantly recovered TP compared to CCl_4 injured group. However, CS-C at 180mg/kg bw showed better recovery from CCl_4-induced hepatic injury in terms of decline in SGOT, SGPT, ALP, TB and increase in TP, compared to CL, CS and CL-C.

Histopathological studies

The histopathological architecture of rat liver tissue showed various hepatocellular damage in CCl_4-treated rats as compared to the normal control group (Figure 3). A single treatment of methanolic fraction at the dose of 180mg/kg bw (CL, CS, CL-C and CS-C), as well as silymarin at 100mg/kg bw restored from the CCl_4-induced liver injury in rats.

The hepatoprotective potential of CL (Coccinia leaf), CS (Coccinia stem), CL-C (Coccinia leaf callus), CS-C (Coccinia stem callus) extracts and CCl_4-induced hepatic toxicity were confirmed by histopathological examination. CCl_4-treated liver showed inflammation, necrosis and disarrangement of cells as compared to the normal control group (figure 3B, 3A). Silymarin (100mg/kg bw) showed normal histological architecture like normal untreated control group Figure 3C, while

single treatment with methanolic fraction of CL, CS, CL-C and CS-C at the dose level of 180mg/kg bw showed significant restoration from inflammation, necrosis, cellular degeneration, vacuolar and vascular congestion (Figure 3D, 3E, 3F, 3G). CS-C treatment showed significantly better recovery from centrilobular vacuolar degeneration than CL, CS and CL-C. The liver undergoes antioxidant stress during

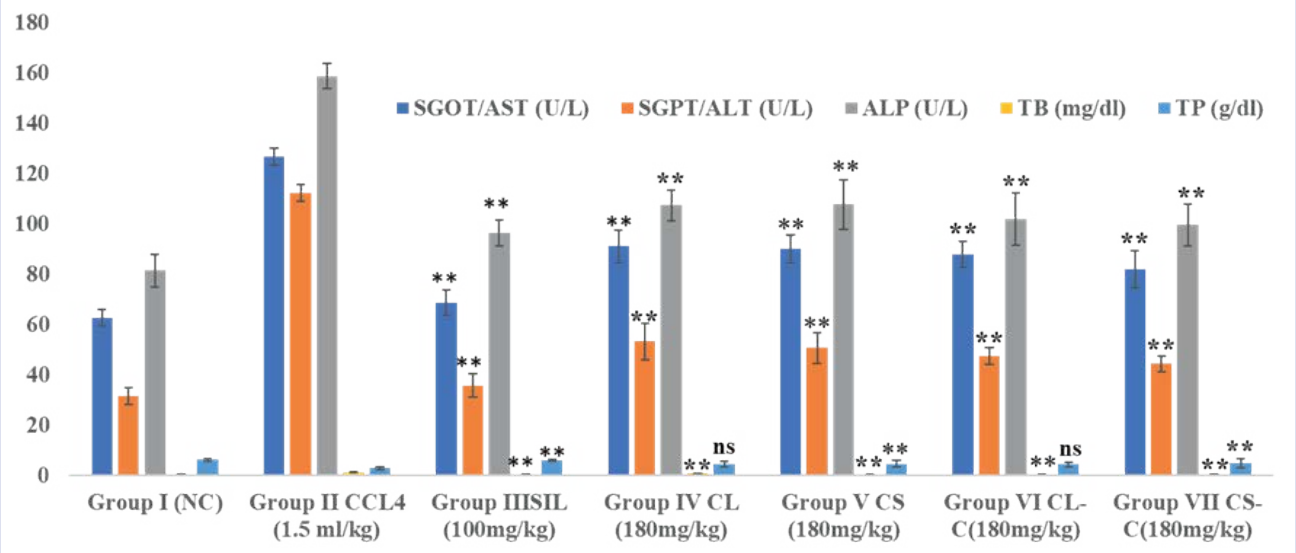

Figure 2: Hepatoprotective potential of CL, CS, CL-C and CS-C.

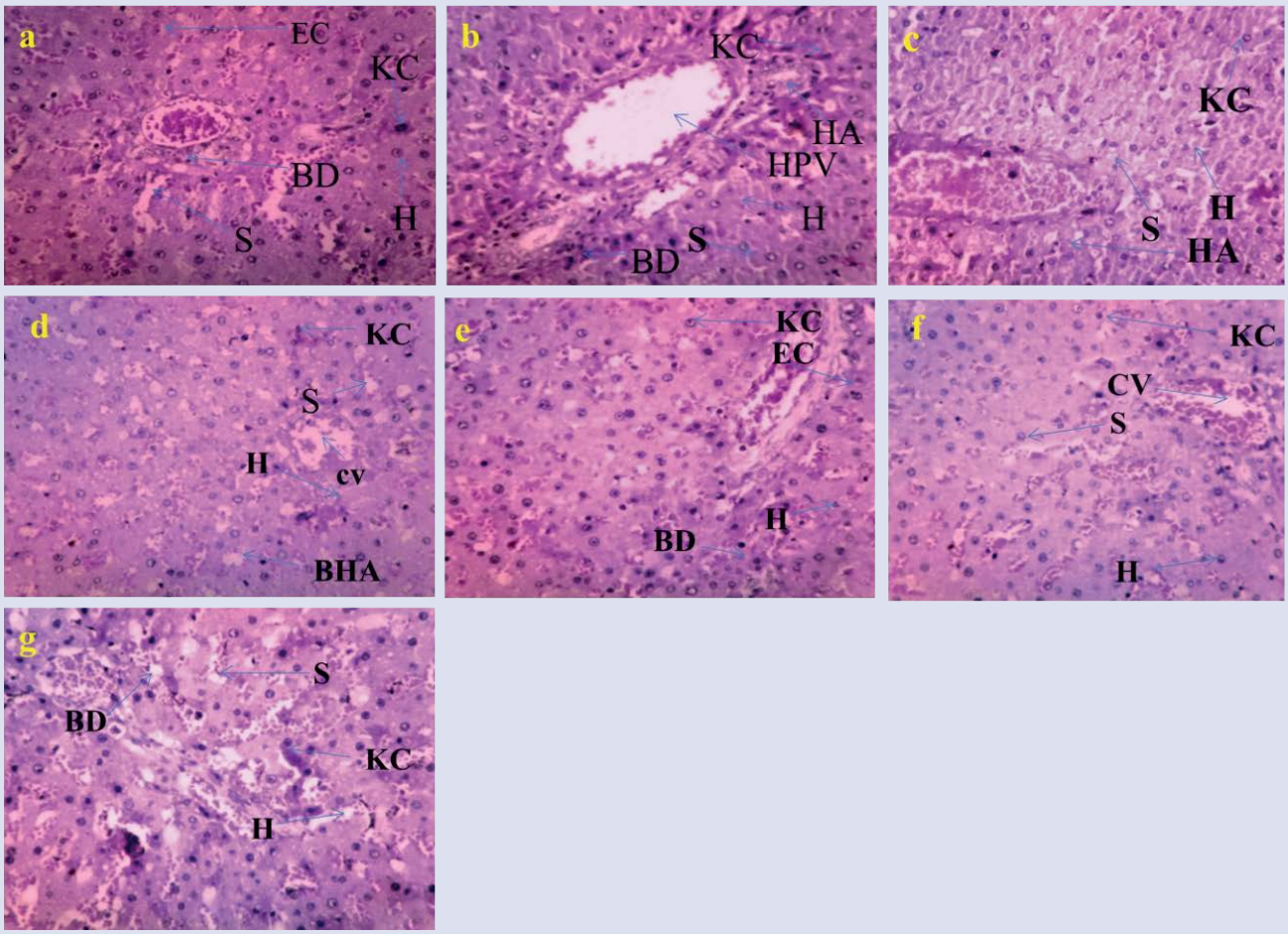

Figure 3: Effects of explants of *Coccinia grandis* plant parts (leaf, stem) and callus (leaf, stem) extracts on structural changes in liver tissue of normal control and treated groups of Albino Wistar rats. (3a) Normal architecture in normal control groups. (3b) A Representative section of the liver from the CCl_4-treated group shows centrilobular vacuolar degeneration of hepatocytes and parenchymal tissue. (3c) Representative section treated with silymarin, a standard drug. Representative sections treated with plant part extracts (3d) CL, (3e) CS, (3f) CL-C and (3g), CS-C. (3c to g) show normal architecture as normal control (3a). **EC, Epithelial cell; KC, Kupffer cell; BD, Bile duct; H, Hepatocytes; S, Sinusoids; HA, Hepatic artery; HPV, Hepatic portal venule, Branch of the hepatic artery; BHA, Central vein; CV.**

Table 1: Selected phytocompounds identified by GC-MS from methanolic extracts of CL, CS, CL-C and CS-C[21].

S. No.	Name of the compounds	Chemical structure	Retention time (RT)	Molecular mass (mg/mol)	Area chromatogram (% content)			
					Leaf (CL)	Stem (CS)	Leaf callus (CL-C)	Stem callus (CS-C)
CL	10-Nonadecanol	$C_{19}H_{40}O$	24.153	284	33.42			
	1-Eicosanol	$C_{20}H_{42}O$	26.287	298	8.66			
	Gamma-Sitosterol	$C_{29}H_{50}O$	26.414	414	7.33			
	Phytyltetradecanoate	$C_{34}H_{66}O_2$	29.353	506	6.12			
	9,10,12,13-Tetra Bromo-octadecanoic acid	$C_{21}H_{36}O_2$	33.322	320	10.63			
	Canadine	$C_{20}H_{21}NO_4$	23.630	339	0.51			
	Protopine	$C_{20}H_{19}NO_5$	23839	353	3.07			
	1-Octadecanol	$C_{18}H_{38}O$	16.195	270	0.48			
	Isopropyl linoleate	$C_{21}H_{38}O_2$	33.029	322	5.63			
	1-Chloroheptacosane	$C_{27}H_{55}Cl$	12.974	414	0.38			
	Neophytadiene	$C_{20}H_{38}$	13.689	278	0.78	0.29		
CS	9-Octadecenamide	$C_{18}H_{35}NO$	21.900	281		85.05	64.36	57.87
	Behenic alcohol	$C_{22}H_{46}O$	24.144	326		0.43		
	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	14.614	270		0.29		
CL-C	9-Octadecenamide	$C_{18}H_{35}NO$	21.896	281			64.36	
	Squalene	$C_{30}H_{50}$	22.050	410			0.31	
	Beta-Amyrin	$C_{30}H_{50}O$	26.983	426			2.29	
	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydro	$C_{18}H_{28}O_3$	14.689	292			4.00	
CS-C	9-Octadecenamid (E)	$C_{18}H_{35}NO$	21.896	210				57.87
	9-Octadecenamide	$C_{18}H_{35}NO$	23.367	281				0.25
	Heneicosane	C21H44	15.333	296				0.50
	Olean-12-en-3-ol, acetate, (3.beta.)-	$C_{32}H_{52}O_2$	26.984	468				1.28
	Gamma.-Sitosterol	$C_{29}H_{50}O$	26.406	414				0.66
	5-(Hydroxymethyl)-2-(dimethoxymethyl) furan	$C_8H_{12}O_4$	7.133	172				4.04

liver injury caused by the accumulation of ROS and active free radicals released due to treatment with CCl_4. The peroxidation of hepatocyte membrane leads to apoptosis and death of hepatocytes, releasing damage-associated metalloproteins (DAMPs) which activate otherwise quiescent hepatic stellate cells (HSC) and Kupffer cells leading to the release of phagocytic macrophages and fibrogens. An intervention of several gene products synthesizing interleukins, chemokines add to the vagary. Therefore, the inhibition of hepatocyte apoptosis could inhibit inflammation, and activation of HSCs and reduce liver fibrosis/injury. The use of methanolic extracts of CL, CS, CL-C, CS-C and Silymarin has evidently reduced hepatic injury by reverting the centrilobular apoptotic vacuolization effectively.

DISCUSSION

In the present investigation evaluation of the hepatoprotective potential of CL, CS, CL-C, and CS-C against CCl_4-induced albino Wistar rats, has been carried out. The results reveal that a single dose (180mg/kg bw) of the given extracts brought out significant hepatoprotective response comparable with silymarin (100mg/kg bw). Silymarin restored levels of serum biochemical markers (SGOT, SGPT, ALP and TB) in rats treated with CCl_4 to the level of normal control due to its anti-inflammatory and antioxidant properties as a polyflavonolignan compound. The administration of CCl_4 at a dose of 1.5ml/kg bw showed hepatopathy due to cytochrome 450 -mediated increased production of active free radicals (CCl_3* and **$OOCCl_3$) leading to a sequence of membrane peroxidation cascade causing damage to hepatocytes along with the leakage of cytosolic enzymes and elevation of total bilirubin in the bloodstream as compared to normal control group. Such results have been reported in the case of other plant extracts too, e.g. by *Linum corymbulosum, Caralluma europea* [6-10-22]. Administration of CCl_4 (1.5ml/kg bw) has been reported to cause degeneration, cellular infiltration, massive coagulation and immense centrilobular necrosis of the liver tissue, hemorrhage, hypertrophy and hyperplasia of Kupffer cells[2]. *Coccinia grandis* is known through ethnobotanical literature to be useful in the treatment of liver diseases[12]. Administration of CL, CS, CL-C and CS-C at a dose of 180mg/kg bw could prevent the CCl_4 (1.5ml/kg bw) induced rise in liver enzyme parameters as compared to normal control by stabilizing the plasma membrane and repairing hepatic injuries through ROS scavenging action. The increased level of TB and decreased level of TP compared to CCl_4-induced injured rats, were significantly restored by the extracts of CL, CS, CL-C and CS-C. Proteins were significantly restored by CS and CS-C compared to CL and CL-C. Histopathological studies also revealed restoration and regeneration of necrotic areas in CCl_4 toxicity-induced injured rats by *C. grandis* extracts. However, CS-C histologically showed a better response to restoration from liver injury than CL, CL-C and CS. The potential of these extracts for restoration from hepatic injury may probably be due to the active antioxidant, anti-inflammatory phytocompounds such as Beta-Amyrin; 1-Chloroheptacosane; Neophytadiene; 1-Octadecanol; Canadine; Protopine; Isopropyl linoleate; Behenic alcohol; Olean-12-en-3-ol, acetate, (3-beta)-; Benzene-propanoic acid,3,5-bis(91,1-dimethylethyl)-4-hydro; 1-Eicosanol; Gamma-sitosterol; Heneicosane and Hexadecenoic acid-methyl ester, 5-(Hydroxymethyl)-2-(dimethoxy methyl) furan[21]. Further investigation is needed for ascribing individual phytocompound as a potential molecule for the restorative/ preventive therapeutic role of hepatic injury.

CONCLUSION

GC-MS profiling revealed the medicinal properties of CL, CS, CL-C and CS-C, with their methanolic extracts showing bioactive compounds such as Beta-Amyrin; 1-Chloroheptacosane; Neophytadiene; 1-Octadecanol; Canadine; Protopine; Isopropyl linoleate; Behenic

alcohol; Olean-12-en-3-ol, acetate, (3-beta)-; Benzene-propanoic acid,3,5-bis(91,1-dimethylethyl)-4-hydro; 1-Eicosanol; Gamma-sitosterol; Heneicosane and Hexadecenoic acid-methyl ester, 5-(Hydroxymethyl)-2- (dimethoxy methyl) furan that could be useful in the restoration from liver injury[21]. The current literature supports the hepatoprotective potential of the reported phytocompounds. This is the first report of *Coccinia grandis* methanolic extracts of calli (leaf, stem) showing hepatoprotective potential against liver injury as no literature could be available on *Coccinia grandis* calli hepatoprotective activity against CCl_4-induced acute toxicity. The present investigation on *Coccinia grandis* leaf, stem and their corresponding calli revealed significant ($p<0.001$) protection of the liver from injury. The histopathological examination further proved the hepatoprotective potential of calli and parent plant parts (leaf, stem) of *Coccinia grandis* against CCl_4-induced toxicity through restoration of degenerated centrilocular necrosed areas. This finding brings out possible new leads such as 9-octadecenamide (an oleamide with antioxidant, mitochondrial dysfunction reducing, sleep-inducing agent) for drug development by pharmaceutical industries.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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