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Research Article
In vitro and *in vivo* Hepatoprotective Activity of Flavonoids Rich Extracts on *Cucumis dipsaceus* Ehrenb. (Fruit)

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Abstract

Background and Objective: Hepatotoxicity caused by hepatoxins is very perilous state of affairs for metabolism of liver. The present study was aimed to investigate hepatoprotective activity on flavonoids rich extracts of (*Cucumis dipsaceus* Ehrenb.) fruits. **Methodology:** *In vitro* hepatoprotective was activity performed on HepG2 cell line by MTT assays for methanolic and water extract to verify cytotoxicity. Acute oral toxicity was plaid on both extracts to know about minimum and maximum dosing by using either sex Wistar albino rats. *In vivo* hepatoprotective activity of both extract was evaluated against CCl_4 induced hepatic damage in liver of Wistar albino rats. Biochemical parameters were studied on blood serum obtained after collecting and clotting of blood form the retro-orbital sinus plexus under mild ether anesthesia and then centrifuged at 3500 rpm for 15 min. *In vivo* antioxidant enzymes activity was evaluated on superoxide dismutase, glutathione, catalase and 2-thiobarbituric acid reactive substances/malondialdehyde (TBARS/MDA) on liver homogenate of rats. Values obtained from every investigation were statistically analyzed by one way ANOVA followed by Tukey's multiple comparison tests using Graph Pad Instat software package. The difference was considered significant if $p<0.05$. **Results:** The results on HepG2 cell line by MTT assays of both extracts were significantly dose dependent. The results of biochemical parameters were found to be significantly normalized from reduced release of these SGOT, SGPT, SALP, billirubin, total protein of CCl_4 treated rats. The results obtained from *in vivo* antioxidant enzymes assays for SOD, CAT, GSH and TBARS/MDA were performed on homogenized part of rat liver after complete dosing were significant than CCl_4 treated group. Histopathological studies were performed on sliced of isolated rat liver after dosing for 7 days. **Conclusion:** The results found from *in vivo* antioxidant activity and hepatoprotective activity showed that both extracts were hepatoprotective and same was supported by histopathological studies on liver of pretreated rat and CCl_4 rats.

Key words: *Cucumis dipsaceus* Ehrenb. (Fruit), antioxidant enzymes, lipid peroxidation, HepG2 cell line, hydrogen peroxide, MTT assay

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Liver is very important and vital organ of the digestion system that all the enzymes which are essential for metabolism are available in liver. So, liver is too impotent for detoxifications of orally administered drugs, which are intentionally or unintentionally absorbed by gastrointestinal tract system and then completely metabolized the ingested drugs and chemicals in liver. Liver is known for clearance house as it contains metabolic enzymes for scavenging and detoxifying the highly reactive species formed by some absurdly action of these enzymes on ingested drugs and chemicals[1]. Liver toxicity and damage is dependended on doses of drugs and chemicals administered by oral route. Paracetamol and CCl_4 are decumbently known to produce centrilobular necrosis due to formation of very reactive toxic metabolite of them[2,3]. Now, both physician and patient eagerly looking for herbal medicine for hepatic protection with least or no side effect for treatment of liver disorders and toxicity[4]. Biomembranes are composed of unsaturated fatty acids and free radicals like to attack on them and results lipid peroxidation of membrane (a hall marks sign of hepatoxicity) and cause reduction in concentration levels of hepatic enzymes and their activity via decrease in fluidity of membrane[5]. This cascade leads to damage to membrane protein which triggers the cell inactivation and finally cell death[6]. So, only antioxidants whether from synthetic and herbal or synergistic action reverse this cascade of destruction of biomembrane by free radicals (from ingested chemical or drugs) through scavenging effect and restored the normal functioning and system of liver[7].

Cucumis dipsaceus (Ehrenb.) is a trailing annual herb (family Cucurbitaceae). It is found in countries like Somalia, Africa, Ethiopia, Kenya, Sudan Tanzania, Uganda and Southern Egypt but now also available in Maruthamalai's forest and its foothills (Western Ghats), District Tamil Nadu (Coimbatore) and Karnataka (Mysore), India. Traditionally, various parts of the herb are used as gastrointestinal diseases, anti-emetic, diarrhoea, stomach pain, hepatitis, gallstone, constipation, rabies, haemorrhoid and meningitis. It has been reported that its fruit possess secondary metabolites like tannin, alkaloids, saponin, flavonoids, resins, steroids and also reported pharmacological activities of extracts of fruit as analgesic, anti-inflammatory activity, cytotoxic activity, antioxidant activity and antimicrobial activity[8]. It is reported that fruit of *Cucumis dipsaceus* Ehrenb. possesses phenolic and flavonoids contents in significant quantity and has strong antioxidant activities which were investigated on chloroform, ethyl acetate, methanolic and water extracts[9]. Other reports showed that flavonoids are responsible for treatment of inflammation of liver damage or hepatotoxicity[10,11]. The ethanolic extracts of *Pterospermum acerifolium* Ster (leaves) possess flavonoids and also protects the hepatic injury introduced by CCl_4 (hepatoxin) via mechanism of free radical scavenging activity and *in vivo* antioxidant activity[12]. Quercetin derivatives as rutin from *Artemisia scoparia* was screened as hepatoprotective activities because it contains free radical scavenging activities against CCl_4 induced hepatotoxicity[13]. Bioactivity guided isolation of flavonoids from methanolic extract of *Equisetum arvense* L. (Equisetaceae) was reported as hepatoprotective activity[14]. The present research work was designed to know about *in vitro* and *in vivo* hepatoprotective activity on flavonoids present in methanolic and water extracts of *Cucumis dipsaceus* Ehrenb. (fruit). This topic was important because hepatitis patient when treated with allopathic medicine, then this therapy twisted side effects and adverse effects and leads to more complications. The cause of liver problems is mostly free radicals and further leads to inflammation. So, biomedicines (plants) which are strong antioxidant and scavenger for free radicals are needed for investigation. This study provide an advance new knowledge for treatment of liver related problems because fruit contains flavonoids, fatty acids and triterpenoids along with micronutrients like minerals which are required for physiological acid-base balance.

MATERIALS AND METHODS

Chemicals: Alkaline phosphatatse (ALP), alanine aminotransferase (ALT/GPT), aspartate aminotransferase (AST/GOT) determination kits were purchased from reckon diagnostics P. Ltd., Vadodars, India. Total bilirubin and total protein determination kits were purchased from Span diagnostics Ltd., Surat, India. All the analytical grade solvents and reagents were used in the study and commercially available chemicals like carbon tetrachloride, 5'-dithio-bis-2-nitrobenzoic acid (DTNB) from (Sisco research laboratories, Mumbai, India.), thiobarbituric acid, trichloroacetic acid. Silymarin powder was obtained from Ranbaxy Laboratories, Delhi, India. Malondialdehyde (MDA) was purchased from Sigma Chemical Company, St Louis, MO, USA. Carbon tetrachloride (CCl_4) was obtained from E Merck, Mumbai, India. All other chemicals and reagents which were used in assays / experiments purchased from reputed vendors of analytical grade.

Plant material: Fruits of *Cucumis dipsaceus* Ehrenb. were collected from Mysore, (Karnataka, India) in November-December 2014. The fruits were identified by

Table 1: Physicochemical parameters and extractive values

Physicochemical parameters	Extractive values
Ash value	5.30% w/w
Acid insoluble ash	2.55% w/w
Water soluble ash	2.15%
Alcohol soluble extractive	20%
Water soluble extractive	35%
Loss on drying	50%
Swelling index	5.00%
Forming index	Less than 25

Dr. Sunita Garg (Head of Raw material, Herbarium and meuseum) at National institute of Science Communication and Information Resources (NSCAIR CSIR), New Delhi 110067. A voucher specimen no. (Ref. No. NISCAIR/RHMD/Consult /2014/2367-147) in the same herbarium.

Phytochemical parameters and extractive values: Phytochemical parameters give very informative knowledge about secondary metabolites present in approximate quantities. So, these parameters were studied likewise, foreign organic matter, swelling index and foaming index, ash values, loss on drying and its extractive values, according to standard procedures as per WHO guidelines and results are shown in Table 1[15,16].

Preparation of crude extracts: Powdered fruits (1.0 kg) of *Cucumis dipsaceus* Ehrenb. after slicing and shade dried were extracted first with n-hexane and then with methanol by soxhlet apparatus refluxing at temperature 35°C. The marc was macerated in water for 24 h with occasional shaking. Afterward concentrated and almost dried with help of rotary vacuum distillation apparatus. The yield found was 120 g. Methanolic extract (100 g) was dissolved in 50 mL methanol and subjected for partitioning with chloroform, ethyl acetate and methanol[9]. All extracts were evaporated and made free from respective solvents by vacuum rotary apparatus.

Phytochemical tests for identification of flavonoids Shinoda test or Mg-HCl reduction test: All extracts were tested by taking 1 mg of extract and was dissolved in 2 mL methanol. Few magnesium turnings were added to it and 2-4 drops of dilute HCl. A pink, orange or red to purple coloration indicate the presence of flavonoids.

Zinc-HCl reduction test: One miligram of each extract was dissolved in distilled water test tube and mixed a pinch of zinc dust was added in to it. Then concentrated HCl was added in it by drop-wise along wall of test tube. Red coloration produced due to presence of flavonoids.

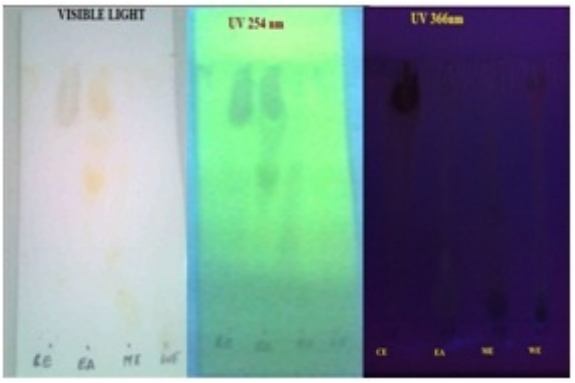

Fig. 1: TLC finger printing of flavonoids

Ferric chloride test: Freshly prepared ferric chloride solution was added drop-wise into each extract solution. Bluish green to black coloration produced due to presence of flavonoids[16-18].

Thin layer chromatography fingerprinting of *Cucumis dipsaceus* Ehrenb.: Thin layer chromatography fingerprinting was performed with methanolic was further partitioned in chloroform, ethyl acetate methanol and water extract to evaluate qualitatively on precoated silica gel aluminium plates $60F_{254}$ (E Merk Germany). Ethyl acetate: Formic acid: GAA: Water (100:11:11:26) was used for evaluating presence of flavonoids in both extract. The development was done by using anisaldehyde-H_2SO_4 reagent (0.5% anisaldehyde in H_2SO_4 solution) as developing agent and observation of spots either in visible and UV light (254 and 366 nm) and Rf values of each spots are shown in Table 2 and Fig. 1[18].

Test animals: Wistar male rats of 180-240 g were procured from the central animal house, ASBASJSM College of Pharmacy, Bela (Ropar)-140111 (Punjab) and required studies were done on them. Polypropylene cages which were large enough and clean were used for animals in room under controlled temperature (22±2°C) and under12 h light and dark cycles with relative humidity (44-55%). Prior a week to studies, all the animals adjusted to laboratory environment. Clean drinking water and a standard rodent pellet diet were provided to the animals according their *ad libitum*. Animal Ethical Committe is constituted in the Institutional. Guidelines were prescribed by the Institutional for care and use of laboratory animals which were strictly followed in accordance. This committee constituted for the purpose of control and supervision of experiments on animals, India.

Table 2: Thin layer chromatography fingerprinting of extracts

Extracts	Rf values	Visible light	UV-VIS light	
			254 nm	366 nm
Chloroform extract	0.88	Grey color	Green blue	Blue-black
Ethyl acetate extract	0.6	Yellow color	Green blue	Blue-black
Methanol extract	0.35	Light yellowish brown color	Green blue	Blue-black
Water extract	0.2	Light brown color	Green blue	Blue-black

Acute oral toxicity: An acute oral toxicity study was performed according to the OECD guidelines for the testing of chemicals, Test No. 423 (OECD, 2001, acute oral toxicity-acute toxic class method). Wistar rats (n = 3) of either sex were selected by a random sampling technique for the acute toxicity study[19].

Hepatoprotective activity evaluation

CCl_4 induced hepatotoxicity: Six groups were made from 36 rats, weighed and then identified by marking with picric acid on their body part accordingly as follows: Group 1 was administered (1 mL kg^{-1} day^{-1} of 1% CMC, p.o.) only the vehicle and served as normal control once daily for 7 days. Group 2 was administered CCl_4 1 mL kg^{-1} (1:1 of CCl_4 in olive oil) i.p daily for 7 days and served as positive control. Group 3 was given CCl_4 1 mL kg^{-1} (1:1 of CCl_4 in olive oil) i.p and administered silymarin 100 mg kg^{-1} orally (p.o.) for 7 days and served as standard control. Groups 4, 5, 6 were administered methanolic and water extracts (6 rats for each extract for each group) of fruit of *Cucumis dipsaceus* Ehrenb. *Cucumis dipsaceus* methanolic extract (CDME) and *Cucumis dipsaceus* water extract (CDWE) at (50, 100 and 200 mg kg^{-1} b.wt.) and (100, 200 and 400 mg kg^{-1} b.wt.,) p.o. respectively were administered along with dose of 1 mL kg^{-1} i.p. of CCl_4 (1:1 of CCl_4 in olive oil) for 7 days)[20-22].

Estimation of biochemical studies: Blood was collected from the retro-orbital sinus plexus after puncturing with capillary tube after mild ether anesthesia. Serum of blood after clotting was separated by centrifugation at 3500 rpm for 15 min. The AST, ALT, ALP, TB and TP in serum was estimated by using reagents kits on biochemistry auto analyser (Merck Lx300, Germany)[20-25].

Preparation of liver homogenate: Liver of the every rat of each group was dissected out after cervical dislocation technique to sacrifice the rats after 24 h from the last treatment to each group. Then livers were washed them with ice cold normal saline solution. Teflon rotary homogenizer was used to made (10% w/v) in saline phosphate buffer (pH 7.4)[26]. The supernatant fraction of homogenate taken after centrifugation at 4000 rpm for 10 min and used for studies of superoxide dismutase (SOD), glutathione peroxidase (GSH), catalase (CAT) and lipid peroxidation by measuring levels of formation of malondialdehyde (MDA)/TBARS.

Estimation of lipid peroxidation and antioxidant enzyme assay on homogenate: The portion of supernatant (microsomal fraction) was used to measure levels the formation of malondialdehyde (MDA) for lipid peroxidation and MDA formed was estimated by using thiobarbituric acid substance. The values of measurements were expressed in nmol of MDA per mg of protein[27]. Superoxide dismutase (SOD) activity was done as described by method and results are expressed as units (U) of SOD activity/mg protein[28]. Catalase (CAT) activity was done as described by method and results are expressed as units (U) of CAT activity mg^{-1} protein[29]. Reduced glutathione (GSH) was done as described by method and results are expressed as GSH formed n mol mg^{-1} protein min^{-1} [30-32].

Liver histoarchitecture study: Formalin solution (10% w/v) was prepared. The dissected liver was fixed in it according to groups. Ethanol (50-100%) was made tissue to dehydrate and then cleaned by xylene. The liver tissue was embedded in paraffin wax. The sections of thickness (5-6 mm) were made and then stained by haematoxylin (H) and eosin (E) dye. The dried sections were then observed under photo microscope for identification of histoarchitecture study liver for cell necrosis, fatty changes, hyaline degeneration, ballooning and infiltration of kupffer cells and lymphocytes[20,21].

***In vitro* hepatoprotective activities of methanolic and water extract on HepG2 cell line:** The methanolic extract was weighed and dissolved in DMSO to obtain 200 mg mL^{-1} stock solution. The non-cytotoxic concentrations of methanolic extract were obtained in HepG2 cells after 24 h of treatment by MTT assay. The highest non-cytotoxic concentration corresponded to 1 mg mL^{-1} of test item. Hepatoprotective potential of methanolic extract was assessed at the concentrations ranging from 0.01-1000 μg mL^{-1} against hydrogen peroxide induced cell injury[33-36].

Statistical analysis: The results of biochemical studies, lipid peroxidation and antioxidant enzyme assay were expressed as Mean±SD (n = 6) and analyzed statistically by one way ANOVA followed by Tukey's multiple comparison test using Graph Pad Instat software demo free trial (InStat3D). The difference was considered significant if $p<0.05$[20].

RESULTS

Hepatoprotective activity of methanolic extract and water extract of *Cucumis dipsaceus* Ehrenb. (Fruit)

Acute oral toxicity: The methanolic extract and water extract of *Cucumis dipsaceus* Ehrenb. (Fruit) were studied for acute oral toxic dose. Methanolic extract causes mortality on 500 mg kg^{-1} dose level. So, only 50, 100 and 200 mg kg^{-1} dose levels were survived with good body functioning. But water extract showed no mortality till 2000 mg kg^{-1} dose level. By this 1/20th, 1/10th and 1/5th i.e. 100, 200 and 400 mg kg^{-1}, p.o. dosing levels were choose for further study. The result of acute oral study showed methanolic extract was not tolerable by rat due to cytotoxic reasons and this extract possesses secondary metabolites which may be responsible for toxicity due to overdosing.

Effect on serum enzymes: Results of biochemical estimation were shown significantly increased ($p<0.05$) in serum SGOT, SGPT, SALP, TB and significantly decreased ($p<0.05$) TP concentrations were in CCl_4 treated groups but the groups pretreated with silymarin or CDME/CDWE significantly decreased ($p<0.05$) in serum SGOT, SGPT, SALP, TB concentration and significantly increased ($p<.05$) TP concentration even due to effect of hepatotoxin CCl_4. It showed the integrity of plasma membrane and restored in status of hepatic tissues (Table 3, 4).

Estimation of lipid peroxidation and antioxidant enzyme assay on homogenate: The results showed significant rise ($p<.05$) in MDA formation level in CCl_4 treated groups but significant fall ($p<0.05$) in MDA formation level with silymarin (100 mg kg^{-1}) and (200 mg kg^{-1} of CDME/400 mg kg^{-1} CDWE) pretreated groups in the presence of CCl_4 in homogenate of liver rats. The SOD, GSH, CAT antioxidant enzymes concentrations were significantly depleted ($p<0.05$) in CCl_4 treated groups but silymarin (100 mg kg^{-1}) and (200 mg kg^{-1} of CDME/400 mg kg^{-1} CDWE) pretreated groups in the presence of CCl_4 showed significantly ($p<0.05$) decrease SOD, GSH, CAT antioxidant enzymes in liver homogenate (Table 5, 6).

Table 3: Biochemical estimation of hepatic biomarker enzymes SGOT , SGPT, ALP, bilirubin and total protein (methanolic extract)

Treatments	SGOT (U L^{-1})	SGPT (U L^{-1})	ALP (KAU)	Bilirubin (mg dL^{-1})	Total protein (mg mL^{-1})
Control	41.33±2.160	054.5±1.871	33.5±1.871	0.535±0.01871	3.375±0.0180
CCl_4 (1 mg kg^{-1})	94.17±2.563[a]	105.0±2.366[a]	78.0±2.828[a]	1.240±0.02317[a]	2.536±0.0594[a]
Silymarin (25 mg kg^{-1}) + CCl_4 (1 mg kg^{-1})	53.50±2.345[a, b]	064.5±2.429[a,b]	37.3±2.422[b]	0.735±0.01871[z,b]	3.289±0.018[b]
CDME crude (50 mg kg^{-1}) + CCl_4 (1 mg kg^{-1})	71.50±3.271[a-c]	082.5±1.871[a-c]	61.8±4.021[a-c]	0.807±0.0216[a-c]	3.130±0.0352[a- c]
CDME crude (100 mg kg^{-1}) + CCl_4 (1 mg kg^{-1})	55.17±3.061[b, c]	065.5±1.9[b, c]	46.5±3.082[a-c]	0.617±0.0356[a-c]	3.183±0.090[a-c]
CDME crude (200 mg kg^{-1}) + CCl_4	41.67±4.719[b, c]	054.7±2.160[b, c]	34.0±3.366[b, c]	0.560±0.011[b, c]	3.319±0.008[b]
F	249.72	312.20	238.65	682.23	251.69
df	5.30	5.30	5.30	5.30	5.30
p	<0.05	<0.05	<0.05	<0.05	<0.05

SGOT: Serum glutamine-oxaloacetic transminase, SGPT: Serum glutamic pyruvic transaminase, ALP: Alakaline phosphates, CDME: *Cucumis dispaceus* methanolic extract. All data are expressed in Mean±SD. The difference was considered significant if $p\leq0.05$, [a]as compared to control, [b]as compared to CCl_4, [c]as compared to silymarin, CCl_4 was administered i.p. and other were given orally

Table 4: Biochemical estimation of hepatic biomarker enzymes SGOT , SGPT, ALP, bilirubin and total protein (water extract)

Treatments	SGOT (U L^{-1})	SGPT (U L^{-1})	ALP (KAU)	Bilirubin (mg dL^{-1})	Total protein (mg mL^{-1})
Control	41.33±2.160	054.5±1.871	33.50±1.871	0.535±0.01871	3.375±0.01802
CCl_4 (1 mg kg^{-1})	94.17±2.563[a]	105.0±2.366[a]	78.00±2.828[a]	1.240±0.02317[a]	2.536±0.05938[a]
Silymarin (25 mg kg^{-1}) + CCl_4 (1 mg kg^{-1})	53.50±2.345[a, b]	064.5±2.429[a,b]	37.30±2.422[b]	0.735±0.01817[a,b]	3.289±0.01808[a,b]
CDWE crude 100 mg kg^{-1} + CCl_4 (1 mg kg^{-1})	54.50±1.871[b, c]	0550±2.191[b, c]	36.17±2.317[b]	0.660±0.04336[a-c]	3.118±0.02026[a-c]
CDWE crude 200 mg kg^{-1} + CCl_4 (1 mg kg^{-1})	45.67±2.805[b, c]	051.5±4.370[b, c]	35.50±1.871[b]	0.565±0.03017[b, c]	3.217±0.01065[a-c]
CDWE crude 400 mg kg^{-1} + CCl_4 (1 mg kg^{-1})	42.00±2.828[b, c]	052.5±2.739[b, c]	34.00±2.191[b]	0.543±0.02338[b, c]	3.321±0.007528[a, b]
F	394.72	512.92	354.80	569.35	726.86
df	5.30	5.30	5.30	5.30	5.30
p	<0.05	<0.05	<0.05	<0.05	0.05

CDWE: *Cucumis dispaceus* water extract, SGOT: Serum glutamine-oxaloacetic transminase, SGPT: Serum glutamic pyruvic transaminase, ALP: Alakaline phosphates, All data were expressed in Mean±SD. The difference was considered significant if $p\leq0.05$, [a]as compared to control, [b]as compared to CCl_4, [c]as compared to silymarin, CCl_4 was administered i.p. and other were given orally

Table 5: Antioxidant enzymes and lipid peroxidation concentration (methanolic extract)

Groups and treatments	SOD (U)	Catalase (U)	GSH (mg g^{-1} tissue)	TBARS/MDA (mg $protein^{-1}$)
Control	1.233±0.1506	2.175±0.1605	5.250±0.2429	3.350±0.1871
CCl_4 (1 mg kg^{-1})	0.326±0.1410[a]	0.557±0.1856[a]	1.367±0.2944[a]	5.560±0.2944[a]
Silymarin (25 mg kg^{-1}) + CCl_4 (1 mg kg^{-1})	1.225±0.1535[b]	2.257±0.1663[b]	4.950±0.1871[b]	3.000±0.2366[b]
CDME crude (50 mg kg^{-1}) + CCl_4 (1 mg kg^{-1})	0.449±0.0171[a, c]	0.686±0.1892[a-c]	2.667±0.3983[a-c]	4.783±0.2563[a-c]
CDME crude(100 mg kg^{-1}) + CCl_4 (1 mg kg^{-1})	1.340±0.1155[b, c]	1.254±0.1039[a, b]	3.950±1.3271[a-c]	4.183±0.2483[a, b]
CDME crude (200 mg kg^{-1}) + CCl_4	1.503±0.2202[a-c]	2.300±0.1190[b]	4.517±0.1515[a, b]	3.033±0.2733[b, c]
F	64.009	158.54	150.31	103.50
df	5.30	5.30	5.30	5.30
p	<0.05	<0.05	<0.05	<0.05

CDME: *Cucumis dispaceus* methanolic extract, SOD: Superoxide dismutare, GSH: Glutathione, TBARS/MDA: 2-thiobarbituric acid reactive substances/malondialdehyde All data were expressed in Mean±SD. The difference was considered significant if $p \leq 0.05$, [a]as compared to control, [b]as compared to, [c]as compared to silymarin, CCl_4 was administered i.p. and other were given orally

Table 6: Antioxidant enzymes and lipid peroxidation concentration (water extract)

Groups and treatments	SOD (U)	Catalase (U)	GSH (mg g^{-1} tissue)	TBARS/MDA (mg $protein^{-1}$)
Control	1.233±0.1225	2.175±0.1605	5.250±0.2429	3.350±0.1871
CCl4 (1mg kg^{-1})	0.326±0.1410[a]	0.557±0.01856[a]	1.367±0.1871[a]	5.560±0.2944[a]
Silymarin (25 mg kg^{-1})+ CCl_4 (1mg kg^{-1})	1.225±0.1535[b]	2.257±0.1663[b]	4.950±0.1789[b]	3.000±0.2366[b]
CDWE crude 100 mg kg^{-1} + CCl_4 (1 mg kg^{-1})	0.845±0.8175[a-c]	0.625±0.1634[a, c]	1.900±0.3869[a-c]	1.900±0.2317[a-c]
CDWE crude 200 mg kg^{-1}+ CCl_4 (1 mg kg^{-1})	1.132±0.0358[b]	1.325±0.1513[a-c]	4.083±0.3204[a-c]	4.083±0.2429[a-c]
CDWE crude 400 mg kg^{-1} + CCl_4 (1 mg kg^{-1})	1.500±0.2297[a-c]	2.321±0.0886[b]	4.767±0.131[b]	4.767±0.2401[b]
F	51.076	167.30	215.72	106.85
df	5.30	5.30	5.30	5.30
p	<0.05	<0.05	<0.05	<0.05

CDWE: *Cucumis dispaceus* water extract, SOD: Superoxide dismutare, GSH: Glutathione, TBARS/MDA: 2-thiobarbituric acid reactive substances/malondialdehyde All data were expressed in Mean±SD. The difference was considered significant if $p \leq 0.05$, [a]as compared to control, [b]as compared to CCl_4, [c]as compared to silymarin, CCl_4 was administered i.p. and other were given orally

Table 7: Non-cytotoxic concentrations of methanolic and water extract

Treatments	Test concentrations (μg mL^{-1})	Cytotoxcity (%)
Untreated		0.00
Quercetin (μM mL^{-1})	5	6.52
Positive control	10	11.92
	50	20.62
Methanolic extract (μg mL^{-1})	0.01	2.50
	0.1	7.26
	1	5.98
	5	11.15
	10	10.73
	50	5.37
	100	3.72
	500	6.26
Water extract (μg mL^{-1})	0.01	3.00
	0.1	4.68
	1	5.02
	5	6.50
	10	7.60
	50	8.30
	100	9.65
	500	15.89

Table 8: Hepatoprotective effect of methanolic and water extract against hydrogen peroxide induced cellular injury

Treatments	Test concentrations	Cell viability (%)
Untreated		100.00
H_2O_2 alone (500 μM)	500	27.80
Quercetin (μM mL^{-1})		
Positive control	10	33.35
	50	43.98
Methanolic extract (μg mL^{-1})	0.01	32.64
	0.1	31.44
	1	29.74
	5	37.29
	10	36.49
	50	33.50
	100	35.36
	500	38.51
Water extract (μg mL^{-1})	0.01	30.20
	0.1	40.80
	1	30.22
	5	28.22
	10	35.22
	50	32.34
	100	31.20
	500	33.32

In vitro **hepatoprotective activities on methanolic and water extract on HepG2 (Human hepatoma) cell line:** The non-cytotoxic concentrations of test item were obtained in HepG2 after 24 h of treatment by MTT assay. The methanolic extract was non-cytotoxic (exhibited <30% cytotoxicity) at all the concentrations tested and slight protection was observed at all the concentrations of methanolic extract. The results are shown in Table 7 and 8.

Liver histoarchitecture study: The normal control rats liver tissues show histoarchitecture as central vein surrounded by

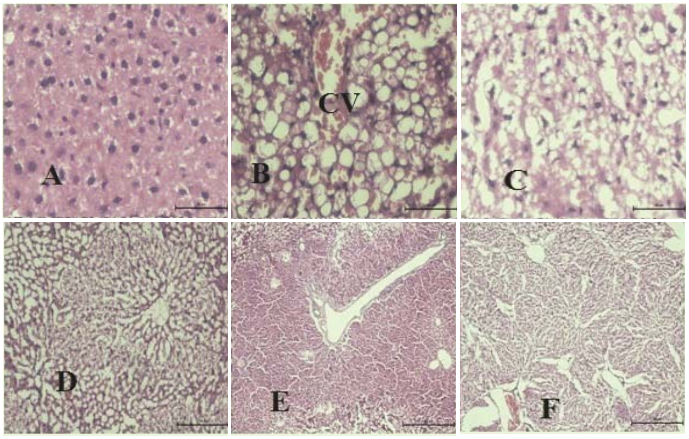

Fig. 2(a-f): Photomicrographs of rats liver section stained with H and E of methanolic extract (Histopathological study)
(a) Liver section of normal control rats, (b) Liver section of CCl_4 treated, (c) CCl_4 and 100 mg kg^{-1} of silymarin treated rat liver section, (d) CCl_4 and 50 mg kg^{-1} of CDME treated rat liver section, (e) CCl_4 and 100 mg kg^{-1} of CDME treated rat liver section and (f) CCl_4 and 200 mg kg^{-1} of CDME treated rat liver section

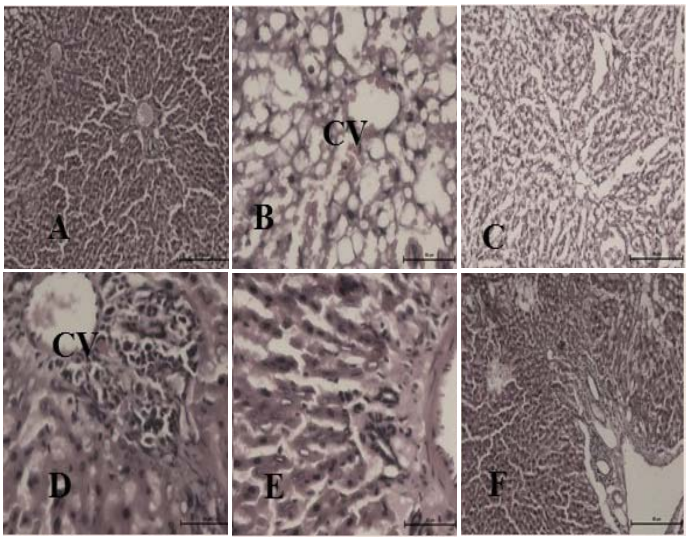

Fig. 3(a-f): Photomicrographs of rats liver section stained with H and E of water extract (Histopathological study)
(a) Liver section of normal control rat, (b) Liver section of CCl_4 treated rat, (c) CCl_4 and 100 mg kg^{-1} of silymarin treated rat liver section, (d) CCl_4 and 100 mg kg^{-1} of CDWE treated rat liver section, (e) CCl_4 and 200 mg kg^{-1} of CDWE treated rat liver section and (f) CCl_4 and 400 mg kg^{-1} of CDWE treated rat liver section

hepatic cord of cells and distinct sinusoidal spaces (normal architecture). Livers sections of animals induced with alone CCl_4 on coarse examination were seen with scattered white areas ascribed to fatty and necrotic changes, massive fatty infiltrations, necrosis, ballooning, degeneration and excess infiltration of lymphocytes were observed. Silymarin (100 mg kg^{-1}) or CDME (200 mg kg^{-1}) and CDWE (200 and 400 mg kg^{-1}) treated groups showed near normal liver architecture, minimal infiltration of lymphocytes and granular degenerative conditions with in both the rat models described in Fig. 2 and 3.

DISCUSSION

The present research study conceals the hepatoprotective activity via strong antioxidant and prophylactic effect of CDME and CDWE against CCl_4 induced hepatotoxicity in rat liver. The liver primarily detoxifies drugs and toxic substances and so

becomes chief mark organ for all possible toxic elements. The CCl_4 is known experimental hepatotoxin used for induction of hepatic damage in rat liver in various animal models. The AST, ALT and ALP are the in vivo antioxidant enzyme present normally in high concentrations in liver but due to oxidative stress as hepatic damage by CCl_4 induction, AST, ALT and ALP leaks into blood circulation and raises serum levels in CCl_4 treated rats and thus predicting the status of liver. Any damage to hepatocytes leaks out as intracellular enzymes to the blood circulation[33]. Significant increase ($p<0.05$) in levels of SGOT, SGPT, ALP, serum bilirubin but the concentration of total protein was significantly decreased in CCl_4 treated group and thus shows severe damage to membrane of hepatocytes, increased permeability and necrosis of hepatocytes. Silymarin treated groups (100 mg kg^{-1}) and prophylactic/pretreated groups with CDME (50, 100 and 200 mg kg^{-1}) and CDWE (100, 200 and 400 mg kg^{-1}) along with CCl_4 showed significantly decreased ($p<0.05$) levels of SGOT, SGPT, ALP, serum bilirubin in liver which was very close to normal control group. Total protein also restored to normal concentration when compares alone CCl_4 treated group[19-24]. Patil *et al.*[37] and Sharstry *et al.*[38] reported that the flavonoids (Quercetin) isolated and characterized from *Amorphophallus paeoniifolius* showed hepatoprotective activity by restoring elevated concentration of SGOT, SGPT, ALP, serum bilirubin and total protein in rat liver against CCl_4 induced hepatic damage. It shows the required repair and stabilization to injury caused to the biomembrane of hepatocytes of liver due to hepatotoxic agent, CCl4[37,38].

The biotransformation of CCl_4 into free radical CCl_3 in liver by cytochrome P_{450} enzymes then reacts with oxygen to form trichloromethylperoxyl radical (CCl_3O_2.). Further, this covalently binds with biomembrane and cellular macromolecules to lipid peroxidation which is responsible for production and leakage of MDA biomarker. This whole gush of biochemical episodes at last causes failure of cellular integrity and hepatic damage. Lipid peroxidation factor along with other free radical biochemical flow during hepatic damage is ultimate oxidative stress. Therefore, a significantly increase ($p<0.05$) in MDA concentration in CCl_4 treated rats. Silymarin, CDME/CDWE produced antioxidant effect and are a treatment which combats oxidative stress and significantly decrease ($p<0.05$) than the CCl_4 treated a group which is possible mechanism of hepatic protection[25]. Ghaffari *et al.*[39] reported that the polyphenolic compounds, flavonoids and terpenoids were responsible for restoring and normalizing increase of MDA due to lipid peoxidation in hepatic damage induced by CCl_4.

The results of SOD, CAT and GSH showed significantly decreased ($p<0.05$) in CCl_4 treated a group than silymarin and CDME/CDWE pretreated groups in the presence of CCl_4. Because superoxide anions (O_2^-), hydroxyl free radicals (OH^-) and hydrogen peroxide (H_2O_2) were responsible for damage to all macromolecular classes in cells and tissues due to induction of CCl_4. The cell itself has capacity to protect oxidative destruction via scavenge, sequester or neutralize Reactive Oxygen Species (ROS) but in presence of silymarin and CDME/CDWE this process of recovery became faster[27-32]. Singhal *et al.*[20] and Surendran *et al.*[40] reported that the concentration of SOD and CAT were potentially restored due to the presence of phenolic and flavonoids in 50% alcoholic extract. So, SOD and CAT is important scavenger of superoxide ion and hydrogen peroxide and provide prevent/protect the macromolecules from oxidative damage. Ayoub *et al.*[41] reported that novel quercetin glycoside with promising hepatoprotective activity isolated from Lobularia libyca was responsible for decreasing the highly elevated concentration of SOD and GSH. It has been also reported that GSH plays important role in detoxifying the reactive toxic metabolites (ROS) mechanism of hepatotoxicity caused by CCl_4[41].

The histoarchitecture structures were further encouraging the effect of hepatoprotective activity due to both extracts[19].

In vitro hepatoprotective effect was studied on HepG2 cells line against H_2O_2 (hepatotoxin as inducing DNA damage is dose dependent and incubation time dependent for HepG2 cell line. These cell lines were vulnerable to induction of DNA damage with low doses of Hydrogen peroxide) by both extracts (CDME and CDWE) at different concentrations (0.01, 0.1, 1, 5, 10, 50, 100 and 500 μg mL^{-1}) showed non cytotoxicity at all concentration and also showed significant cell viability (%) in presence of hepatotoxic agent H_2O_2. These extracts also indicate the restoration and protection of hepatic cells from toxic effect due to (·OH) hydroxyl free radical produced by hydrogen peroxide for oxidative stress. The correlation between (antioxidant activity and lipid peroxidation) *in vivo* hepatoprotective and *in vitro* hepatoprotective (non cytotoxic study) on CDME and CDWE showed percent cytotoxicity study on HepG2 cells reveals the standard drug quercetin in different μM mL^{-1} concentration as positive control showed significant dose dependent percent cytotoxic effect. The CDME firstly showed dose dependent percent cytotoxic effect up to concentration 10 μM mL^{-1} but afterward CDME showed dose independent percent cytotoxic effect on HepG2 cells. This change gave indication for extra toxicity (contrary effect) due to presence of some other phytoconstituents which by increasing dose decreased the percent cytotoxic effect on HepG2 cells. The

CDWE showed significantly dose dependent percent cytotoxic effect on HepG2 cells which revealed the presence of phytoconstituents showed more cytotoxic effect as its concentration increased. The pattern of quercetin and CDWE results verify the presence of same type of compound must be present in CDWE and in CDME some other. Antioxidant activity in vivo hepatoprotective study showed CDME and CDWE both showed dose dependent effects through SOD, CAT, GSH and TBARS against CCl_4 induced hepatic damage in liver. Both CDME and CDWE showed excellent scavenging effect on free radical and restored effect of lipid peroxidation on biomembrane of hepatocytes. This showed that both extracts have phytoconstituents (polyphenolic constituents like flavonoids and related glycoside) which act as strong antioxidant for scavenging (·OH) hydroxyl free radical. So, these extracts showed hepatoprotective potential towards hepatoxicity due to hepatotoxicity by H_2O_2[33-36,39,42].

CONCLUSION

It is concluded that the both CDME and CDWE extracts possess hepatoprotective activity due to strong antioxidant activity and scavenging effect on free radical by serum hepatic biomarker enzymes and isoenzymes or their synergistic of phytophenolic active secondary metabolites present in both extracts and *in vivo* hepatic enzymes. This study will be further explored for structure elucidation by instrumental spectrophotometer and advance hyphenated techniques and also this judgment may possibly lead to use of isolated compound as intoxicating hepatoprotective remedy.

SIGNIFICANCE STATEMENT

This study discovers the possible synergistic effect of protein, carbohydrates, amino acid, minerals, vitamins, phenolic and flavonoids, fatty acid (Linoleic acid) and saponin (cucurbitacin) from fruit of *Cucumis dipsaceus* Ehrenb. These can be beneficial for scavenging the free radicals produced by hepatoxin (CCl_4) which induces hepatic damage to hepatocytes of rat liver, similar to viral hepatitis disease. Thus, it is a new theory of combination with micronutrient and secondary metabolites which may be the best arrived remedy for hepatitis treatment.

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