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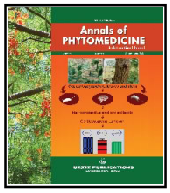

Original Article : Open Access

Ameliorative effect of lemongrass (*Cymbopogon flexuosus* Nees ex Steud.) W. Watson and celery (*Apium graveolens* L.) against CCl_4 induced oxidative stress and acute hepatotoxicity in rats: An *in vivo* assessment

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Abstract

Indian herbs like lemongrass and celery are rich in phytochemicals and antioxidants that can provide therapeutic effects. Our study aimed to formulate detoxifying drink variants and evaluate their hepatoprotective potential against carbon tetrachloride-induced toxicity (0.5 ml/kg/day i.p) in rats. Standard drink (SD) was prepared and from this SD variants were prepared by adding different ratios of 0.5%, 1.0% and 1.5% of lemongrass powder (LG5, LG10 and LG15) and celery powder (CL5, CL10 and CL15), respectively. Fifty-six rats were divided into eight treatment groups where group V-VIII received SD variants having Indian herbs. Groups II was treated as negative control which received CCl_4 only and Group III was treated as positive control and received silymarin at a dose of 100 mg/kg/day + CCl_4. Rats pretreated with detoxifying drink variants significantly (p=0.05) lowered the pathogenesis of CCl_4-induced hepatotoxicity. Group VIII receiving the CL15 variant showed the results in line with the positive control group. CL15 pretreatment ameliorated CCl_4 induced toxicity by reducing plasma liver function parameters like AST (64.9%), ALT (71.7%), ALP (57.4%), total bilirubin (1.65 mg/dl) and increasing plasma protein content (6.01 g/dl) as compared to CCl_4 group. CL15 also significantly (p=0.05) improved antioxidant enzyme activity (SOD, GPx and CAT) and increased GSH concentration while increasing lipid peroxidation (MDA) in liver homogenate.These findings recommend that lemongrass and celery can strategically be used to formulate plant-based therapeutic drugs for liver health.

1. Introduction

The liver is the major site for the metabolism of environmental toxicants, and thus is susceptible to the toxic overload of xenobiotic and oxidative stress. In the liver, toxin-metabolizing enzymes either neutralize or eliminate xenobiotics from the body. However, in case of incomplete metabolism, toxic substances are transformed into nucleophiles and electrophiles which are highly reactive substances (Seif, 2016; Kiran *et al*., 2019). Carbon tetrachloride (CCl_4) is a xenobiotic causing hepatotoxicity in human beings and animals. The liver has a biological capacity to regenerate damaged tissues and trigger antioxidant enzymes that protect against CCl_4-induced toxicological manifestation. However, if toxicity exceeds the capacity of the liver to repair, it causes necrosis and cell damage (Khan *et al*., 2012; Tlili *et al*., 2016).

Hence, the use of indigenous plants to assist the liver in detoxification remains an indispensable option.WHO estimates three-quarters of the world currently rely on traditional medicines for therapeutic needs (Danciu *et al*., 2018; Jyothilekshmi *et al*., 2020; Yadav *et al*., 2021). Nowadays, polyherbal formulations made up of active herbal ingredients are gaining popularity for their synergistic pharmacological effect in curing various oxidative stress triggered diseases (Jyothilekshmi *et al*., 2020; Duraisami *et al*., 2021).

Lemongrass (*Cymbopogon flexuosus* Nees ex Steud.) W. Watson is a grassy plant belonging to the Poaceae family. It is used in nutritional, pharmaceutical and flavouring industries (Lonkar *et al*., 2013; Jiang *et al*., 2017). Lemongrass contains several polyphenolic compounds including gallic acid, isoquercetin, quercetin, rutin, catechin and tannic acid which attributes to its high antioxidant activity (Somparn *et al*., 2018). Besides, lemongrass has an abundant reservoir of essential oil like citral, a mixture of geranial and neral; myrcene, citronella, limonene, geraniol, nerol, α-terpineol and eugenol responsible for its distinct flavour and aroma (Lonkar *et al*., 2013; Jiang *et al*., 2017; Li *et al*., 2017). The essential oil and citral in lemongrass are reported to induce phase II drug-metabolizing enzymes like NAD(P)H: quinine oxidoreductase 1 (NQ1), Glutathione-S-transferase (GST) and UDP-glucuronosyl transferase (UGT). Induction of phase II enzymes results in a protection against toxicity and chemical carcinogenesis (Li *et al*., 2017).

Celery (*Apium graveolens* L.) is an aromatic plant belonging to the Apiaceae family. Being a good source of vitamin C helps prevent the

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free radical damage that triggers the inflammatory cascade. Besides, celery is a rich source of bioactive compounds like flavonoids, alkaloids, steroids, glycosides, phenols, volatile oils and sesquiterpene alcohols. The extract of celery contains flavonoids like apigenin, apiin, luteolin and kaempferol and polyphenolic compounds like caffeic acid, protocatechuic acid, chlorogenic acid and coumaric acid (Danciu *et al*., 2018). Among these, apigenin is the main flavonoid and p-coumaric acid is the main phenolic acid present in the crop (Danciu *et al*., 2018; Golubkina *et al*., 2020). Studies have revealed that the administration of extracts of celery has a hepatoprotective effect on chemical-induced liver toxicity (Shivashri *et al*., 2013; Tanasawet *et al*., 2017).

Silymarin (*Silybum marianum*) is a hepatoprotective, rich in phyto-chemicals, *viz*., flavonoids, phenols, alkaloids, terpenoids, steroids and glycosides. It has efficient antioxidant and anti-inflammatory properties (Kiran *et al*., 2019; Saber *et al*., 2020). The present study, therefore, aimed at formulating detoxifying drink variants that can be used as a therapeutic food-based approach to prevent liver diseases and reduce oxidative stress.

2. Materials and Methods

2.1 Plant material

For the formulation of detoxifying drink variants, lemongrass (*C. flexuosus* Nees ex Steud.) W.Watson-Krishna variety and celery (*A. graveolens* L.) celery 1 was procured from PAU, Ludhiana Punjab, India.

2.2 Detoxifying drink preparation

Standard drink (SD) was prepared by using different proportions of the plant materials using basil leaves, mint leaves, ginger, cucumber and cinnamon powder (Figure 1). Different ingredients for SD were selected based on their previously reported detoxifying ability. Further using SD as a base, various detoxifying drink variants were formulated using lemongrass and celery in different proportions. For lemongrass and celery variants, washed, blanched (90ºC for 5 min) and oven-dried (45ºC for 8 h), lemongrass and celery leaves that were later grounded into a powder were used. The lemongrass and celery powder were added at 0.5%, 1.0% and 1.5% for different variants of lemongrass (LG5, LG10 and LG15) and celery (CL5, CL10 and CL15), respectively in SD, as shown in Table 1. Rats were fed with 0.5% and 1.5 % respective drinks after concentrating them 2.5 times. The concentration of detoxifying drinks fed to rats (mg/10 ml) is given in Figure 2.

2.3 Phytochemical analysis

Total phenolic content (TPC) and total flavonoid content (TFC) were determined using Mathur and Vijayvergia (2017) method. Total antioxidant activity was determined by scavenging 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical and expressed as % inhibition (Tadhani *et al*., 2007).

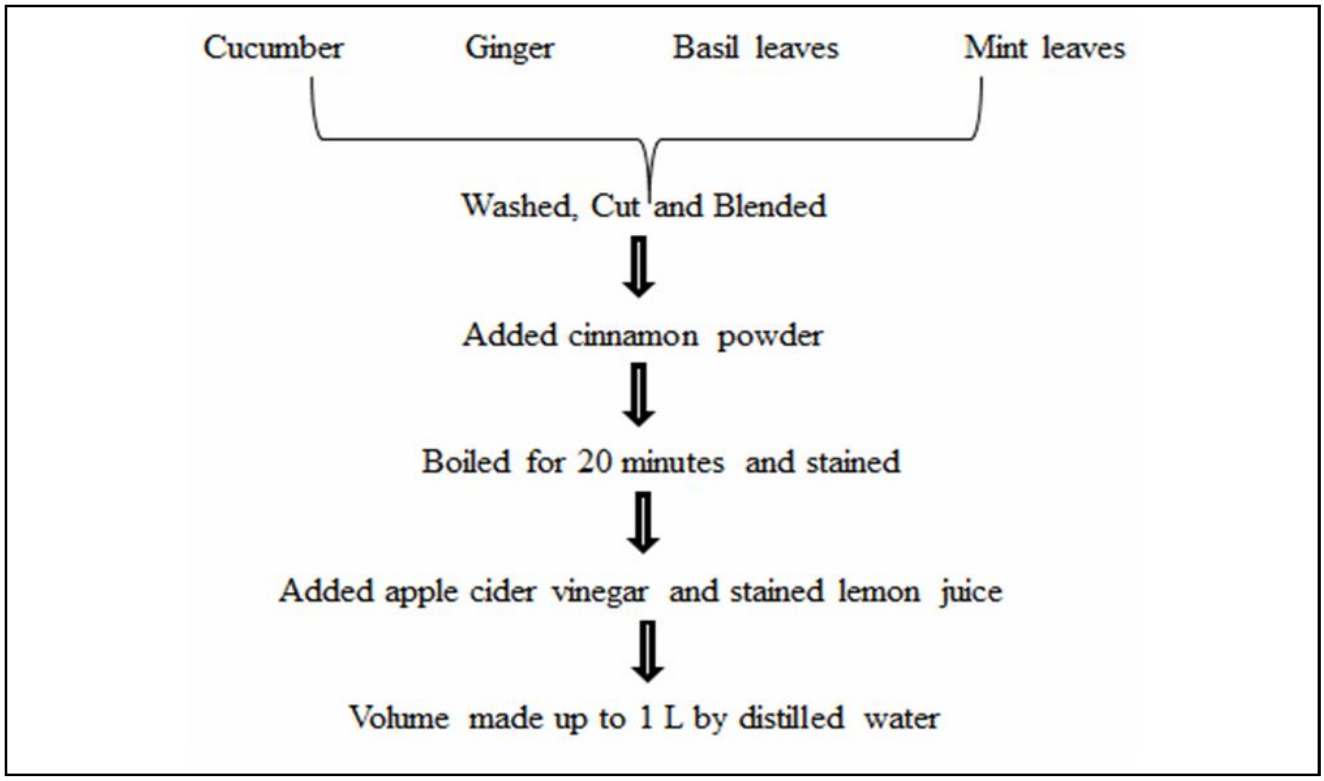

Figure 1: Procedure to prepare standard detoxifying drink (SD).

2.4 Animal study

Male wistar albino rats weighing 200-300 g were procured from NIPER, Punjab. Rats were accommodated in the plastic cages with free access to food and water *ad libidum*. The animals were maintained under standard conditions (22 ± 3ºC, 12 h dark/12 h light, RH 35-55%). The rats were acclimatized for 15 days before the experiment.

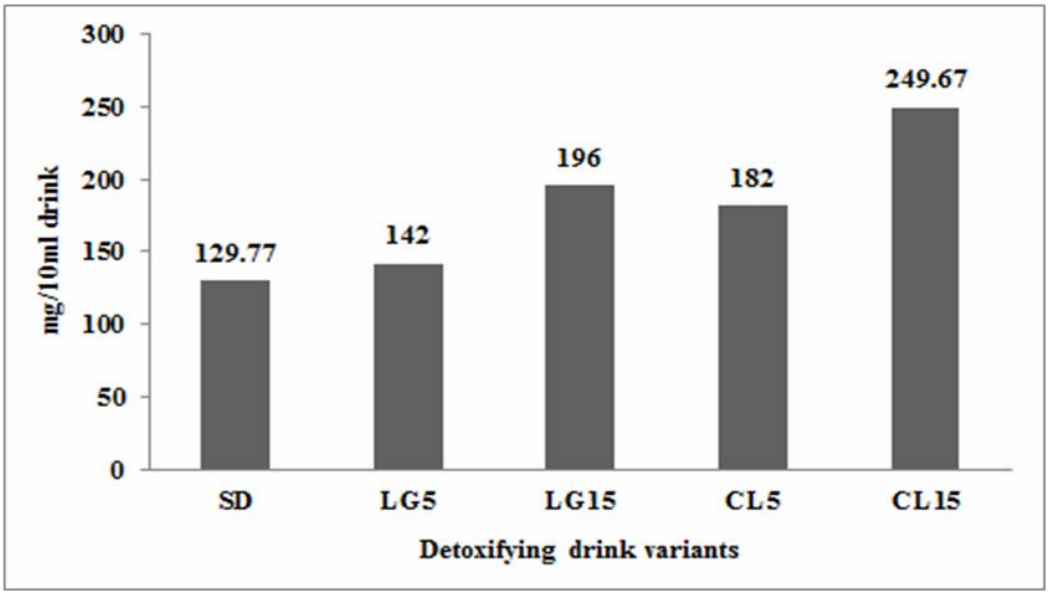

Figure 2: Dry matter concentration of detoxifying drink variants (mg/10 ml of drink).

Table 1: Ingredients and their proportion in detoxifying drink variants

Ingredients	SD	LG5	LG10	LG15	CL5	CL10	CL15
Cucumber	200 g	200 g	200 g	200 g	200 g	200 g	200 g
Lemon juice	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml	20 ml
Ginger sliced	5 g	5 g	5 g	5 g	5 g	5 g	5 g
Apple cider vinegar	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
Basil leaves	20 g	20 g	20 g	20 g	20 g	20 g	20 g
Mint leaves	20 g	20 g	20 g	20 g	20 g	20 g	20 g
Cinnamon powder	5 g	5 g	5 g	5 g	5 g	5 g	5 g
Lemongrass powder (LG)	-	0.5%	1.0%	1.5%	-	-	-
Celery leaves powder (CL)	-	-	-	-	0.5%	1.0%	1.5%
Final volume made by distilled water	1000 ml	1000 ml	1000 ml	1000 ml	1000 ml	1000 ml	1000 ml

SD: Standard drink; LG5: 0.5% of lemongrass powder; LG10: 1% of lemongrass powder; LG15: 1.5% of lemongrass powder; CL5: 0.5% of celery powder; CL10: 1% of celery powder; CL15: 1.5% of celery powder.

2.5 Experimental protocol and procedure

56 rats were divided into eight groups with 7 rats in each group; Group I: Normal control received distilled water; Group II: Negative control received CCl_4 only (0.5 ml/kg i.p)(Shenoy *et al.*, 2001); Group III: Positive control received silymarin 100 mg/kg/day and CCl_4; Group IV: Received standard drink (SD) and CCl_4; Groups V and VI: Received 0.5% and 1.5% lemongrass detox drinks (LG5 and LG15), respectively along with CCl_4; Groups VII and VIII: Received 0.5% and 1.5% celery detox drink (CL5 and CL15), respectively along with CCl_4. The detoxifying drink variants were given at 2 ml/100 g BW/day for 4 consecutive weeks.The combination of doses selected for feeding was based on the highest organoleptic acceptability of the detoxifying drink variants (LG5 and CL5) and the highest nutritional, phytochemical and antioxidant activity that was found in LG15 and CL15 drink variants.Animals were sacrificed 24 h after the injection of CCl_4.

2.6 Feed and water intake

Both feed and water intake were recorded for three days before starting the dose and for three days before sacrificing the animals.

2.7 Bodyweight and organ weight

We recorded the weight of the rats once before starting the dose and before sacrificing the animals. The organ was weighed and relative organ weight (per 100 g body weights) was calculated.

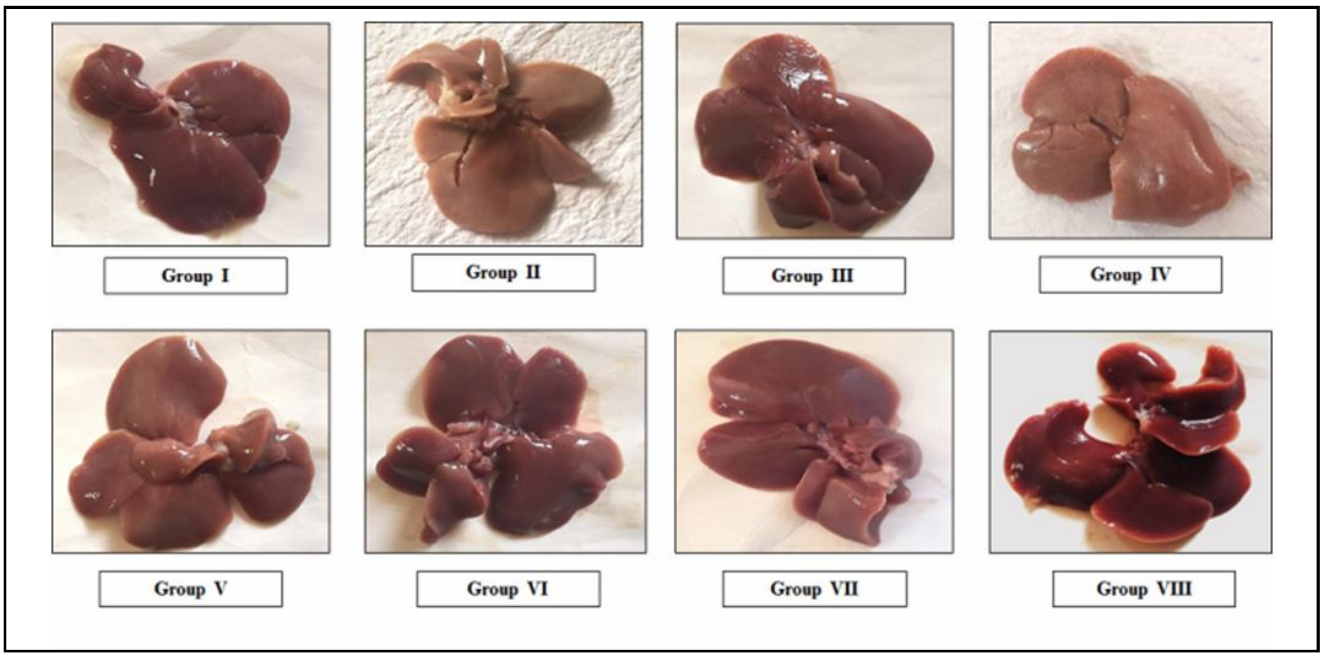

Figure 3: Liver samples of various experimental rat groups.

2.8 Liver function test

At the end of the experiment, animals fasted overnight and were sacrificed by cervical dislocation.The protocol for the study was approved by the Institutional Animal Ethics Committee of College of Veterinary Science, GADVASU, Ludhiana, India (Approval No. GADVASU/2018/IAEC/46/17). All experiments were carried out following the guidelines laid down by CPCSEA, New Delhi, India. Blood was collected through the cardiac puncture into heparin tubes and plasma was separated by centrifugation at 3000 rpm for 15 min at 4°C and stored at – 20°C.The plasma liver function parameters for hepatotoxicity like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct and indirect bilirubin and albumin and total protein were analyzed by assay kits (Oscar medicare private limited, New Delhi).

2.9 Determination of antioxidant status

The liver organ of all sets of animals was excised, washed, and weighed. 10% w/v tissue homogenate was prepared with a tissue homogenizer (IKA® T25 digital ultra Turrax®) in ice-cold PBS with EDTA. SOD activity was estimated by Madesh and Balsubramaniam (1997) method. GPx was performed by the method of (Hafemann *et al*., 1974) based on the oxidation of GSH in the presence of hydrogen peroxide. CAT activity was assayed by measuring the rate of decomposition of hydrogen peroxide at 240 nm as described by Aebi (1983). Total protein was determined using bovine serum albumin (BSA) as a standard (Lowry *et al*.,1951) and GSH activity was measured based on reaction with DTNB (Beutler *et al*., 1963). Lipid peroxidation was performed by the method of Buege and Aust (1978) using a molar extinction coefficient of pure MDA as 1.56×10^5.

2.10 Statistical analysis

The data were expressed as mean ± SD with seven replicates in an animal study and triplicates in phytochemical analysis. Data were analyzed by analysis of variance (ANOVA) at a significance level of <0.05 using JAM 10.0.1 software. Tukey-Kramer HSD test was applied in the animal study for determining the significance.

3. Results

3.1 Phytochemical components and antioxidant activity in standard drink and its variants

The TPC (mg GAE/100 ml of drink) of a standard drink (SD) was 59.15 ± 0.30 while the TPC at 0.5%, 1.0% and 1.5 % was 72.08 ± 0.95, 81.18 ± 1.16 and 104.75 ± 5.00, respectively in lemongrass drink variants and 91.43 ± 2.72, 120.30 ± 2.00 and 136.48 ± 3.10 in 0.5%, 1.0% and 1.5 % of celery drink variants. While TFC (mg QE/ 100 ml of drink) of SD was 4.48 ± 0.17 and the TFC at 0.5%, 1.0% and 1.5 % of lemongrass drink variants was 10.03 ± 0.63, 15.10 ± 0.23 and 21.86 ± 0.74, respectively and 12.27 ± 0.36, 20.92 ± 0.90 and 30.20 ± 1.78 in 0.5%, 1.0% and 1.5 % of celery drink variants. The DPPH assay of the drink showed percent-dependent free radical scavenging activity and was highest in CL15. The total antioxidant activity expressed as % inhibition of DPPH radical ranged from 21.37 ± 1.26 to 40.76 ± 1.02 in lemongrass drinks and 35.17 ± 0.95 to 56.81 ± 1.60 in celery drink variants. The result suggests standard detoxifying drink with celery has higher phenols and flavonoids than lemongrass and a corresponding better free radical scavenging capacity.

3.2 Effect of detoxifying drink on feed and water intake of rats

Intraperitoneal injection of CCl_4 did not affect the feed and water consumption as it was administered 24 h before the sacrificeas depicted in Table 2. Instead, the rats pretreated with detoxifying drinks showed a reduction in post-study feed and water intake. This decrease in feed and water intake was in line with the percentage of lemongrass and celery powder, as CL15 and LG15 showed higher reductions than CL5 and LG5, respectively.

Table 2: Effect of detoxifying drink variants on water and feed intake of normal and CCl_4 hepatotoxicity-induced in rats

Group	Treatment	Water intake (ml)		Mean difference	t-ratio	Feed intake (g/100 g BW/day)		Mean difference	t-ratio
		Pre-study	Post-study						
I	Normal control	14.07 ± 0.02	14.23 ± 0.23	0.14	1.19 (0.82)	7.72 ± 1.29	7.87 ± 2.22	0.15	0.09 (0.53)
II	CCl_4	11.29 ± 0.13	11.13 ± 0.24	- 0.15	-1.82 (0.10)	7.44 ± 1.01	7.09 ± 0.97	-0.35	-6.32 (0.01)
III	Silymarin + CCl_4	11.24 ± 0.08	11.33 ± 0.17	0.07	1.23 (0.82)	6.70 ± 0.83	6.27 ± 0.87	-0.43	-7.00 (0.009)
IV	SD + CCl_4	13.76 ± 0.12	12.09 ± 0.15	- 1.68	-33.05 (0.0005)	7.19 ± 1.02	6.61 ± 0.95	-0.57	-4.78 (0.02)
V	LG5 + CCl_4	14.25 ± 0.08	11.76 ± 0.11	- 2.49	-23.00 (0.0009)	7.30 ± 0.75	6.98 ± 2.46	-0.32	-0.21 (0.42)
VI	LG15 + CCl_4	12.10 ± 0.06	9.22 ± 0.18	- 2.88	-30.37 (0.0005)	7.72 ± 1.25	6.73 ± 1.16	-1.00	-14.00 (0.002)
VII	CL5 + CCl_4	11.61 ± 0.06	9.61 ± 0.13	- 2.01	-28.12 (0.0006)	7.29 ± 0.74	6.62 ± 0.62	-0.67	-9.16 (0.005)
VIII	CL15 + CCl_4	12.58 ± 0.14	10.23 ± 0.23	- 2.34	-13.85 (0.002)	7.16 ± 0.49	5.97 ± 0.27	-1.20	-9.08 (0.006)

Table 3: Effect of detoxifying drink variants on the percentage change in body weight and liver weight of normal and CCl_4-induced hepatotoxic rats

Group	Treatment	Initial weight	Final weight	% increase in BW	Absolute liver weight (g)	Relative liver weight (%)
I	Normal	252.57 ± 39.19	335.57 ± 51.79	32.88 ± 1.45	9.07 ± 1.40	2.70 ± 0.03
II	CCl_4	259.86 ± 38.31	333.00 ± 50.33	**28.09^b ± 0.66**	**11.09^a ± 1.49**	**3.34^a ± 0.20**
III	Silymarin + CCl_4	250.29 ± 36.05	333.14 ± 47.99	33.10^a ± 0.82	9.45^a ± 1.36	2.84^d ± 0.09
IV	SD + CCl_4	245.14 ± 32.39	317.29 ± 42.20	29.41^b ± 0.96	10.03^a ± 1.23	3.16^b ± 0.05
V	LG5 + CCl_4	241.29 ± 37.25	312.00 ± 49.01	29.27^b ± 1.03	9.63^a ± 1.26	3.10^{bc} ± 0.09
VI	LG15 + CCl_4	264.57 ± 40.72	341.00 ± 52.21	28.91^b ± 0.88	10.14^a ± 1.49	2.98^{cd} ± 0.07
VII	CL5 + CCl_4	244.00 ± 29.66	314.86 ± 37.27	29.08^b ± 0.94	9.07^a ± 0.95	2.89^d ± 0.06
VIII	CL15 + CCl_4	256.00 ± 37.67	328.57 ± 48.57	28.34^b ± 0.64	9.34^a ± 1.33	2.85^d ± 0.07

All values represent mean ± SD, n = 7 animals; values with the same superscript along the column are not significantly different ($p \leq 0.05$). Bold values in 2nd row indicates a significant difference ($p \leq 0.05$) between CCl_4 group (Group II) and normal control (Group I) Tukey-Kramer HSD test has been applied for the given parameters.

3.3 Effect of detoxifying drink on body weight gain and relative liver weight

The body weight change (%) and relative liver weight of the animals in the control and experimental group are represented in Table 3. The % body weight change was 32.88% in normal control (Group I) which was significantly ($p \leq 0.05$) reduced in Group II (receiving CCl_4 only) to 28.09%. Group III (receiving silymarin + CCl_4) significantly ($p \leq 0.05$) increased the percentage change in body weight as compared to the negative control Group II. But, there was a reduction in the weight of rats that received detoxifying drink variants (LG5, LG15, CL5 and CL15) as compared to the normal control. The relative liver weight of the normal control group was 2.70%. Group II (receiving CCl_4 only) significantly ($p \leq 0.05$) increased the relative liver weight (3.34%) as compared to Group I. Among the groups those received detoxifying drink variants of lemongrass and celery, there was a significant ($p \leq 0.05$) reduction in relative liver weight in all groupsas compared to the negative control group (Group II). At higher concentrations of LG15 and CL15, the relative liver weight was in line with the silymarin effect.

3.4 Effect on liver function tests

The effect on AST, ALT and ALP after induction of liver injury by i.p of CCl_4 is shown in Table 4. Administration of CCl_4 significantly ($p \leq 0.05$) increased the level of liver function parameters in plasma. CCl_4 hepatotoxic group also witnessed a significant ($p \leq 0.05$) decrease in plasma protein and albumin as compared to the normal group. On the other hand, these altered liver function biomarkers were completely restored ($p \leq 0.05$) near the normal range by pre-treatment of lemongrass and celery detoxifying drinks. The experimental test levels lean towards bioequivalence in pharmacokinetics with silymarin treatment. Besides, a significant ($p \leq 0.05$) increase in the levels of direct and total bilirubin was observed in rats exposed to CCl_4 injection compared with the normal control as shown in Table 4. All variants of drink along with silymarin suppressed ($p \leq 0.05$) the level of bilirubin.

3.5 Effect on antioxidant status in liver homogenate

CCl_4 administration showed a significant ($p \leq 0.05$) reduction in the antioxidant enzyme activity of SOD, GPx and CAT as shown in Table 5. In the CCl_4 intoxicated Group (II), GPx, SOD and CAT were depleted to 1.80 µg/mg, 6.70 U/mg and 3.72 U/mg, respectively. While the groups received the celery and lemongrass detoxifying

drinks augmented the activity of SOD, GPx and CAT. Better results were seen in groups receiving higher concentrations (LG15 and CL15) which were closer to the effect shown by silymarin. Oxidative stress parameters (reduced GSH and MDA) are also shown in Table 5. CCl_4 treated rats showed a significant ($p \leq 0.05$) increase in MDA and a reduction in GSH level. All celery and lemongrass drink variants reduced the MDA and increased GSH levels significantly ($p \leq 0.05$). Per cent inhibition of MDA was highest in silymarin, followed by CL15 and LG15, keeping CCl_4 treated group as the baseline.

Table 4: Effect of detoxifying drink variants on plasma activities of liver function enzymes of normal and CCl_4-treated hepatotoxic rats

Group	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (mg/dl)	DB (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
I	Normal	37.89±4.48	23.26±4.70	253.55±8.16	0.70±0.02	0.27±0.03	6.68±0.42	3.76±0.09	2.92±0.39
II	CCl_4	147.56^{a}±12.31	142.75^{a}±20.09	691.03^{a}±25.60	1.65^{a}±0.17	0.66^{a}±0.03	4.63^{d}±0.16	2.68^{d}±0.03	1.95^{c}±0.15
III	Silymarin + CCl_4	44.25^{e}±3.95	32.87^{e}±4.42	276.29^{e}±6.13	0.92^{d}±0.13	0.28^{d}±0.03	6.13^{a}±0.24	3.30^{a}±0.21	2.82^{a}±0.26
IV	SD + CCl_4	107.75^{b}±5.85	107.33^{b}±14.01	397.65^{b}±8.22	1.33^{b}±0.16	0.54^{b}±0.04	5.14^{c}±0.24	2.75^{d}±0.11	2.39^{b}±0.18
V	LG5 + CCl_4	91.10^{c}±5.78	72.52^{c}±5.03	321.21^{c}±9.17	1.31^{b}±0.02	0.49^{b}±0.05	5.68^{b}±0.18	2.86^{cd}±0.14	2.82^{a}±0.19
VI	LG15 + CCl_4	76.41^{d}±3.63	50.26^{d}±1.98	304.58^{cd}±14.53	1.28^{bc}±0.01	0.42^{c}±0.02	5.97^{ab}±0.20	3.00^{bc}±0.21	2.97^{a}±0.27
VII	CL5 + CCl_4	78.07^{d}±3.85	50.63^{d}±5.39	305.71^{cd}±7.73	1.24^{bc}±0.15	0.41^{c}±0.02	5.96^{ab}±0.21	3.086^{abc}±0.12	2.87^{a}±0.30
VIII	CL15 + CCl_4	51.79^{e}±2.10	40.71^{de}±2.85	293.86^{de}±8.55	1.09^{cd}±0.11	0.30^{d}±0.02	6.01^{ab}±0.20	3.206^{ab}±0.07	2.81^{a}±0.19

All values represent mean ± SD, n = 7 animals; values with the same superscript along the column are not significantly different ($p \leq 0.05$). Bold values in 2nd row indicates a significant difference ($p \leq 0.05$) between CCl_4 group (Group II) and normal control (Group I).

Tukey-Kramer HSD test has been applied for the given parameters; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TB: total bilirubin; DB: direct bilirubin.

Table 5: Effect of detoxifying drinks on enzymatic parameters in liver homogenate of normal and CCl_4-intoxicated rats.

Group	Treatment	SOD (U/mg protein)	GPx (U/mg protein)	CAT (U/mg protein)	MDA (nmoles/ mg protein)	% inhibition	GSH (µmole/g liver tissue)
I	Normal	2.89±0.07	12.74±0.14	6.73±0.09	0.94±0.08	-	11.16±0.22
II	CCl_4	1.80^{f}±0.08	6.70^{f}±0.21	3.72^{f}±0.08	3.87^{a}±0.08	0	7.82^{f}±0.22
III	Silymarin + CCl_4	2.88^{a}±0.04	12.19^{a}±0.26	6.80^{a}±0.09	1.02^{g}±0.07	73.51	11.01^{a}±0.16
IV	SD + CCl_4	2.14^{e}±0.04	7.97^{e}±0.15	4.53^{e}±0.08	3.51^{b}±0.03	9.26	8.22^{e}±0.19
V	LG5 + CCl_4	2.33^{d}±0.04	8.70^{d}±0.12	5.17^{d}±0.08	2.91^{c}±0.05	24.63	8.74^{d}±0.13
VI	LG15 + CCl_4	2.64^{b}±0.04	10.25^{b}±0.18	6.22^{b}±0.08	1.93^{e}±0.07	50.14	9.78^{b}±0.17
VII	CL5 + CCl_4	2.56^{c}±0.04	9.89^{c}±0.13	5.33^{c}±0.04	2.58^{d}±0.08	33.31	9.22^{c}±0.15
VIII	CL15 + CCl_4	2.83^{a}±0.05	12.21^{a}±0.11	6.72^{a}±0.05	1.30^{f}±0.08	66.48	10.98^{a}±0.21

All values represent mean ± SD, n =7 animals; values with the same superscript along the column are not significantly different ($p \leq 0.05$). Bold values in 2nd row indicates a significant difference ($p \leq 0.05$) between CCl_4 group (Group II) and normal control (Group I).
Tukey-Kramer HSD test has been applied for the given parameters; SOD, superoxide dismutase; GPx, Glutathione peroxidase; CAT, catalase; MDA, malondialdehyde; GSH, reduced glutathione.

4. Discussion

The majority of previous studies have focused only on the formulation of detoxifying drinks. There is a visible research gap in terms of nutritional evaluation of these drinks and their protective effect against hepatotoxicity.

We used CCl_4 to induce toxicity and investigated the hepatoprotective potential of lemongrass and celery. Several plant-derived chemical compounds have been identified as a potential therapeutic strategy to maintain the normal homeostasis of the antioxidant enzyme system and suppress oxidative stress (Seif, 2016; Sarhadynejad *et al*., 2016). CCl_4 intoxication in rats is widely used to study necrosis and steatosis of the liver. CCl_4 causes acute toxicity in the liver *via* bioactivation of the cytochrome P450 oxygenase system. Trichloromethylperoxyl and trichloromethyl are two highly reactive free radicals generated by CCl_4. These free radical by-products attack hepatocytes and cause fatal injury by covalently binding on proteins or unsaturated lipids and thus, causing lipid peroxidation (Popovic *et al*., 2006; Khan *et al*., 2012; Tlili *et al*., 2016).

The increased oxidative stress results in depletion of antioxidant enzymes and non-enzymatic antioxidants in the body as well as augments lipid peroxidation that further stimulates the formation and propagation of lipid radicals, and generates by-products like ketones, alcohols, aldehydes, and ethers (Jiang *et al*., 2017). Consequently, oxidative stress and ROS overload can also be

assessed by measuring these by-products like malondialdehyde (MDA), assessing depletion of non-enzymatic parameters like GSH and reduced activity of antioxidant enzymes like SOD, CAT and GPx.

In this study, results showed that CCl_4 significantly ($p<0.05$) reduced the levels of all antioxidant enzymes (SOD, CAT and GPx) and GSH in rats. Identically, these deleterious changes were similar to the results of previous studies on CCl_4 toxicity (Popovic *et al*., 2006). Celery and lemongrass administration reversed the lipid peroxidation symptoms and increased antioxidant enzyme activity; this may be attributed to the higher antioxidant capacity of celery and lemongrass due to the polyphenols and flavonoids present. Additionally, improved activities of hepatic antioxidant enzymes are correlated with the reduction of lipid peroxides.

The pharmacological efficacy of celery is documented in various previous studies. In earlier studies, celery extract (125, 250 and 500 mg/kg) decreased MDA and increased GPx activity in the cortex and striatum in anxiolytic rats (Tanasawet *et al*., 2017) and reversed the deleterious toxic effect of doxorubicin-induced hepatotoxicity (Kolarovic *et al*., 2010) and acetaminophen (Shivashri *et al*., 2013). In another study, extracts of celery leaves and roots demonstrated effective hydroxyl and DPPH radical scavenging capacity against CCl_4 induced-hepatotoxicity (Popovic *et al*., 2006). Apiin, a flavonoid in celery is suggested to possess scavenging capacity thus, reduces MDA level and significantly enhancing antioxidant enzyme activities (Li *et al*., 2014). Moreover, apigenin, another flavonoid in celery has been reported to up-regulate the gene expression of phase II detoxification enzymes like GST and UGT. GST and UGT aid in the elimination of xenobiotics by conjugating the electrophiles and nucleophiles with glucuronidase and the addition of glutathione (Popovic *et al*., 2006; Danciu *et al*., 2018).

Similarly, earlier studies have reported the hepatoprotective effect of polyherbal formulations (Jyotilekshmi *et al*., 2020). Likewise, the therapeutic potential of lemongrass in its various forms like dried powder, tea, juice, oil and solvent extracts in reversing the effect of toxicity has been documented. This effect is due to the presence of bioactive compounds and nutrients present in lemongrass that scavenger ROS and ameliorate drug toxicity (Jiang *et al*., 2017; Li *et al*., 2017). Krishna variety of lemongrass contains maximum essential oil, ascorbic acid and total chlorophyll as compared to other varieties which contributes to its high antioxidant status (Lonkar *et al*., 2013). Lemon grass oil and citral at 400 mg/kg in a study have shown to increase detoxification enzymes like NQ1 and UGT activity (Li *et al*., 2017). In another *in vivo* study on benzo(a)pyrene-induced carcinogenesis, lemongrass essential oil and citral reduced MDA and increased antioxidant activity of SOD and CAT activities thus, combating and reversing the toxic effect of benzo(a)pyrene (Jiang *et al*., 2017).

Also, in this study, CCl_4 administered rats showed a decline in body weight gain and an increased liver weight, depicting deprived growth and CCl_4 induced toxicity (Khan *et al*., 2012). Liver weight was close to normal control in silymarin and other experimental rats. However, there was a decline in weight gain of celery and lemongrass administered rats. Celery helps reduce the accumulation of lipid in adipose tissue and prevents high fat-induced obesity. The anti-adipogenic property of celery is due to its capacity to inhibit lipid accumulation in adipose cells *via* activation of the AMPK enzyme (Adenosine monophosphate-activated protein kinase) which in return deactivates the enzymes involved in lipid synthesis. Furthermore, celery helps weight loss by preventing hepatic steatosis and up-regulating liver antioxidant enzymes (Cho *et al*., 2020). Studies have also shown that lemongrass leaf extracts inhibit α-amylase activity and lipase activity present in the digestive system. Thus, lemongrass can function as herbal medicine to cure obesity (Ongmali *et al*., 2017).

Liver function tests are screening tools that are widely acceptable and effective in detecting hepatic dysfunction. These enzymes are released into circulation after an autolytic breakdown and the quantity of release in the blood is proportional to the severity of hepatopathy (Popovic *et al*., 2006; Khan *et al*., 2012). Hyperbilirubinaemia occurs when the liver produces more bilirubin than it can metabolize, which further damages the hepatic cells and obstructs the excretion of bilirubin. The plasma protein, albumin and globulin content depict the liver's capacity to biosynthesis molecules in the hepatic (Seif, 2016).

The administration of celery and lemongrass detoxifying drink variants significantly ($p<0.05$) attenuated the CCl_4 induced alteration in liver function tests and reverse the atrocious effect of CCl_4. In a study, the aqueous leaf extract of lemongrass (200, 30 and 400 mg/kg) reduced the serum AST, ALT and ALP levels as well as attenuated bilirubin in hepatotoxic rats which was otherwise increased by paracetamol-induced hepatotoxicity (Ozims *et al*., 2017). Lemongrass and citral have also shown similar results against acetaminophen-induced toxicity (Li *et al*., 2017). Celery is also shown in previous studies to improve the serum protein and albumin levels in alloxan-induced diabetic rats (Mans and Aburjai, 2019). Thus, lemongrass and celery detoxifying drinks can be commendably used in formulating therapeutic plant-based drugs to prevent liver diseases and reduce oxidative stress.

5. Conclusion

The risks of chronic liver diseases and their progression can be attenuated by enhancing the body's indigenous antioxidant defense system. This study provides experimental evidence and justifies traditional claims that lemongrass and celery can be effectively used in the prevention or treatment of liver diseases. The detoxifying drink variant CL15 was as effective as silymarin (herbal liver drug) in protecting the liver injury against CCL_4 induced toxicity.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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