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Hepato and Nephro protective potentials of lyophilized juice of *Citrus reticulata* L. fruit against paracetamol induced toxicity in rats

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Abstract: This study scrutinized the phytochemical composition, curative, hepato and nephro protective effect of different doses of lyophilized juice of *Citrus reticulata* fruit against paracetamol induced toxicity. Phytochemical screening and RP-HPLC analysis were conducted to quantify total polyphenols and flavonoids respectively. For evaluation of *in vivo* curative and protective effects, thirty six rats were randomly divided into six groups. In first four groups 1, II, III and IV paracetamol 75mg/kg,i.p,150mg/kg,250mg/kg and 500mg/kg p.o doses of lyophilized juice were administered to rats respectively. Blood samples were withdrawn at 0, 24, 48 and 72 hours in paracetamol treated rats. For screening of hepato and nephro protective effect Group V and VI were fed on lyophilized juice (250mg/kg and 500mg/kg p.o) for seven days and on 8th day blood samples were collected at 0,24,48 and 72 hours. Hepatic and renal biomarkers were monitored. Phytochemical analysis revealed the presence of total polyphenols (20.7±0.3GAE mg/g) and flavonoid contents (21.2±0.4QE mg/g). RP-HPLC also confirmed the presence of Myricetin, Quercetin, and Kaempferol in fruit juice. The lyophilized juice at 500mg/kg dose have shown profound decrease in paracetamol induced elevated serum levels of liver and kidney functions, which suggests a possible therapeutic role of its constituents in hepatic and kidney malfunctions.

Keywords: RP-HPLC, hepatoprotective, polyphenols, myricetin, paracetamol, *Citrus reticulate.*

INTRODUCTION

The liver is an imperative organ in the human body that maintains hemostasis, metabolism of carbohydrates, proteins, and fats, and detoxification. Nowadays, unhealthy food and lifestyle can lead to liver diseases. Liver diseases caused by drugs are more common than those that occur naturally. Drugs can also harm the liver and kidneys (Talluri *et al.*, 2018; Chariyakornkul *et al.*, 2022). Paracetamol is an important NSAID used to treat pain and has the potential to cause liver and kidney failure. Liver diseases caused by drugs are more common than those that occur naturally. The performance of Currently available treatments for hepatic injuries has remained unsatisfactory (Shams *et al.*, 2024). In the past few decades, drug discovery has been marvelously progressed but patients are still in dire need of effective remedies with least side effects. Since the beginning of human civilization, herbal remedies have had a long ritual usage. The large population of developing countries uses natural remedies to treat health-related problems as compared to conventional therapies (Ansari and Husain, 2012).One of the important medicinal plant example is *Citrus reticulata* L. Which belongs to the family Rutaceae and commonly known as mandarin. Mandarin is a rich source of vitamins C and A, protein, flavonoids, dietary fibers, pectin, and essential minerals including calcium, magnesium, phosphorus, and potassium (Shorbagi *et al.*, 2022). Mandarins phytochemicals i.e. phenols, flavonoids and terpenoids, act as insect repellent. Mandarins are laxative and improve digestion. Peel of fruit actions as a skin moisturizer and used to treat skin diseases (Musara *et al.*, 2020). Keeping in view the health benefits of *C. reticulata* present study was designed. Phytochemical screening and quantification of flavonoids were performed by using RP-HPLC. Additionally, the hepato- and nephro-protective potential of lyophilized juice of *C. reticulata* fruit against paracetamol-induced toxicity model in rats was also evaluated.

MATERIALS AND METHODS

Collection and authentication of plant material

The fruit was collected from the medicinal garden of Institute of Pharmaceutical Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan and authenticated by a taxonomist and a specimen voucher (GC.Herb.Bot.3423) was obtained. After collection, fruit juice was extracted and preserved.

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Lyophilization of fruit juice

Before extraction of juice, Mandarin fruits were inspected for any spoilage. Fruits were washed with water to remove pesticide residues and dust particles. Fresh mandarins were cut into pieces before processing. Power-operated commercial juice extractor (squeeze) was used to extract fruit juice (Abou-Arab *et al.*, 2018). A small quantity of juice (3ml) was poured into vials for proper lyophilization with a micropipette and the sample was weighed along with the vial. Sample vials were freezed at -40 to -60°C for 24 hours in lyophilizer (Labfreez F10 series, China). For the primary and secondary phases of freeze drying, different temperature and pressure levels were adjusted. Once the lyophilization process was completed, vials were weighed to determine water loss for each sample. The final weight of freezed dried juice was on average 18% of the original weight. Freezed dried samples were finally stored at -18°C (Corrêa-Filho *et al.*, 2019; Indelicato *et al.*, 2023).

Determination of total polyphenol contents

Slinkard and Singleton (1997) method was used to estimate total polyphenol contents (TPC). The standard calibration curve was drawn by using Gallic acid as standard. A volume of 0.2mL of both the sample and standard solutions was pipetted into separate test tubes, followed by the addition of 0.2mL of Folin-Ciocalteu's phenol reagent. After 4 min of thorough stirring 1mL 15% Na2CO3 solution was added to test tubes. Samples were kept for two hours at room temperature and absorbance was measured at 760 nm. All reagents are present in blank solution except the analyte. The standard curve of Gallic acid was drawn by using a linear regression equation for the determination of total polyphenol content as a milligram of gallic acid equivalent. The total polyphenol content was calculated using the following formula (Waheed *et al.*, 2024):

Total phenols = (Gallic acid equivalents in µg/mL) × (volume of extract in mL) / (sample weight in grams)

Determination of total flavonoid contents

The Chang *et al.* (2002) method was used to estimate total flavonoid contents (TFC). The standard calibration curve was drawn by using Quercetin as standard. 0.1mL of 1M potassium acetate, 0.1mL of 10% aluminum nitrate solution, and 4.6mL of distilled water were combined with 0.2mL of each of the sample and standard solutions in separate test tubes. The test tubes were incubated for 45 minutes at room temperature.

Similarly, blank solutions were prepared without analyte, and at 415 nm absorbance was measured. The standard curve of quercetin was drawn by using a linear regression equation for the determination of total flavonoid content as a milligram of quercetin equivalent. Total flavonoid contents were calculated using the formula (Waheed *et al.*, 2024):

Total flavonoids = (Quercetin equivalents in µg/mL) × (volume of extract in mL) / (sample weight in grams)

Simultaneous quantification of flavonoids by using RP-HPLC

Flavonoids present in the lyophilized juice of *C. reticulata* fruit were estimated through RP-HPLC (Sultana and Anwar, 2008).

Chromatographic conditions and sample analysis

The analysis was performed using a reverse phase high performance liquid chromatography system (RP-HPLC) model (LC- 10A, Shimadzu, Kyoto, Japan) equipped with vacuum degasser (DGU-20A5), two quaternary pumps (LC-20AT), auto sampler (SIL-20AC), column oven (CTO-20AC) and UV detector (SPD-M20A). The detector was operated in a sensitivity range of 0.05AUFS. Output of the range was 15mV and the data acquisition was performed with LC-10 software. The sample was prepared by dissolving 10mg of lyophilized juice in 2 ml of methanol. The stock solution of myricetin, quercetin, and kaempferol was prepared by dissolving 10 mg of each flavonoid in 100 ml of deionized water individually. All the samples as well as the standard solution were filtered by using 0.45µm PTFE syringe filters.

Each sample/standard solution (20µL) eluted through the column C18 Merck (5µm, 4.6 x 250 mm particle size) – using an isocratic mobile phase containing TFA 50%: acetonitrile and methanol 50%, at a flow rate of 1.0ml/min. The temperature of the column was maintained at 30°C and detection was carried out using DAD detector, at wavelength 360nm. The peaks obtained were compared to the standard using retention time. The concentrations of the standards in the different extracts can be calculated using the formula (Saleem *et al.*, 2014):

$$\text{Concentration of unknown} = \frac{\text{Peak area of sample solution}}{\text{Peak area of standard solution}} \times \text{Conc. of standard}$$

Evaluation of in-vivo hepatoprotective activity

Maintenance of animals

For *in-vivo* experimentation, thirty-six healthy male Wister rats were purchased from the University of Veterinary and Animal Sciences, Lahore. Each rat with an average weight of 250g was selected. All animals were kept at the animal house of the Institute of Pharmaceutical Sciences, University of Veterinary and Animal Sciences, Lahore, and acclimatized for two weeks. Animals were fed on water and pelletized feed *ad libitum.*

Ethical considerations

All experimental manipulations involved in current research conformed to the guiding principle for research involving animals as recommended by the declaration of Helsinki and the guiding principles in the care and use of animals and also approved by the institutional Ethical Review Committee (NO.DR /597) of UVAS.

Experimental procedure

For evaluation of *in-vivo* curative and hepato- and nephroprotective effect of lyophilized juice of *C. reticulata* fruit, thirty six rats were randomly divided into six groups (n=6). In the first four groups I, II, III, and IV Paracetamol 75mg/kg, i.p, 150mg/kg, 250mg/kg and 500mg/kg p.o doses of lyophilized juice were administered to rats respectively. Blood samples were withdrawn at 0, 24,48and 72 hours in paracetamol-treated rats. For evaluation of hepato- and nephro-protective effect Group V and VI were fed on lyophilized juice of *C. reticulata* fruit (250mg/kg and 500mg/kg p.o) for seven days and on 8th day blood samples were collected at 0,24,48 and 72 hours. Hepatic and renal biomarkers were monitored. Zero-hour blood samples were withdrawn from all animals in the groups just before administration of paracetamol 75mg/kg, i.p. Blood samples were withdrawn at 24, 48 and 72 hours. The effect of paracetamol was observed on different enzymes and biochemical parameters (Serum ALT, AST, ALP, LDH, Uric acid, Creatinine and Urea) at 0, 24, 48 and 72 hours. The toxic dose for Paracetamol was calculated according to standard protocol (Chomchai *et al.*, 2011). Serum enzymes (ALT, AST, ALP, LDH) and serum biochemicals (uric acid, urea and creatinine) were estimated by using Randox® kits.

STATISTICAL ANALYSIS

The results were represented as mean ± S.E. Data was analyzed by using one way analysis of variance (ANOVA) followed by Dunnet multiple comparison tests to find out the significant difference between groups. . Mann Whitney test was used to analyze statistical difference between two groups. P value< 0.05 considered as a significant difference.

RESULTS

Determination of total polyphenol contents and total flavonoid contents

The result of total polyphenol contents in lyophilized juice was measured as 20.7±0.3 GAE mg/g. Total polyphenols were estimated by using the linear regression equation y = 0.0739x - 0.0177 (R^2 = 0.9982) which was derived from the gallic acid standard curve.

The result of total flavonoid contents in lyophilized juice was measured as 21.2±0.4 QE mg/g. Total flavonoids were estimated by using the linear regression equation y = 0.0021x + 0.0163 (R^2 = 0.9977) which was derived from the quercetin standard curve.

Estimation of flavonoids by using RP-HPLC

The successful separation can be observed in the representative HPLC chromatograms of a standard mixture, of lyophilized juice of *C. reticulata* fruit in fig. 1 and 2. Three flavonoids were simultaneously identified as myricetin, quercetin and kaempferol, and their concentration in lyophilized juice of *C. reticulata* fruit were measured as 0.29µg/g, 0.21µg/g and 0.05µg/g respectively.

Acute toxicity studies

No mortality was observed with 150,250 and 500mg/kg of lyophilized juice of *C. reticulata* fruit. It is observed that animals remain healthy and active after receiving these doses. Behavioral changes such as tremors, traction, loss of appetite, lacrimation, salivation, hair erection, diarrhea mortality, and other symptoms of toxicity were observed in rats for 15 days (Alema *et al.*, 2020).

Evaluation of hepatoprotective activity

The effects of the lyophilized juice of *C. reticulata* fruit were studied on the activity of enzymes (ALT, AST, LDH and ALP), and biochemical parameters i.e. serum Urea, uric acid and creatinine. The lyophilized juice of *C. reticulata* at the dose of 150,250 and 500 mg/kg p.o were administered to Paracetamol-treated rats. Blood sampling was done at, 24, 48, and 72-hour intervals. Zero-hour samples were taken before the administration of lyophilized juice. The results are shown in table 1, 2, 3 and 4. For hepatoprotective action, 250 and 500 mg/kg p.o doses of lyophilized juice of *C. reticulata* were administered for 7 days; on 8th day zero hour blood samples were withdrawn and Paracetamol 75mg/kgi.p was administered, further blood sampling was done at 24,48,72 hours post-administration of Paracetamol. The results are shown in table 5 and 6.

DISCUSSION

Pakistan is sanctified with rich medicinal plants and a large population depends on traditional remedies. These traditional remedies are composed of phytoconstituents responsible for treating many ailments. However, to rationalize these herbal preparations, thorough phytochemical characterization and biological screening are obligatory. In the current study, *C. reticulata* fruits were collected from the IPS medicinal garden in October 2022. Fruits were washed and juice was extracted.

Juice was lyophilized in order to preserve its color, flavor, nutrients and thermolabile phytoconstituents (Indelicato *et al.*, 2023). Lyophilized juice percentage yield is 18%. Lyophilized juice was subjected to thorough phytochemical and biological screening. Quantitative phytochemical analysis revealed the presence of flavonoids (21.2 ± 0.4 QE mg/g and polyphenols (20.7 ± 0.3 GAE mg/g.) in lyophilized fruit juice.

Simultaneous quantitative estimation of flavonoids in lyophilized juice of *C. reticulata* was performed by using RP-HPLC. It was observed that flavonoids i.e. Myricetin (0.29µg/g), Quercetin (0.21µg/g), Kaempferol (0.05µg/g) were present in moderate amounts in lyophilized fruit juice.

Table 1: Effect of paracetamol (75mg/kg i.p) on serum enzyme and biochemical parameters in rats.

Serum levels (Mean ± S.E)				
Enzymes and biochemicals	0hour	24hour	48hour	72hour
ALT (U/L)	13.80 ± 0.40	48.09 ± 0.28*	100.60 ± 0.88*	200.90 ± 0.37**
AST (U/L)	13.14 ± 0.41	95.66 ± 0.88*	120.00 ± 1.15*	225.06 ± 1.55**
LDH(U/L)	205.00 ± 2.88	518.00 ± 1.15*	523.00 ± 4.04*	529.66 ± 5.54**
ALP(U/L)	65.23 ± 1.63	173.30 ± 2.40*	182.60 ± 3.17*	291.00 ±3.21**
Urea (mg/dl)	7.00 ± 0.11	9.50 ± 0.17*	10.63 ± 0.21*	16.16 ± 0.44**
Uric acid (mg/dl)	9.32 ± 0.06	9.40 ± 0.20	9.80 ± 0.10*	11.50 ± 0.76**
Creatinine (mg/dl)	1.13 ± 0.08	0.7 ± 0.03	2.70 ± 0.05*	2.76 ± 0.08**

*P < 0.05, **P < 0.01 significant difference, ALT: Alanine transaminase*; AST:* Aspartate aminotransferase; LDH: Lactate dehydrogenase; ALP: Alkaline phosphatase, n=6, Dose of Paracetamol =75mg/kg i.p

Table 2: Effect of lyophilized juice of *C. reticulata* (150mg/kg) fruit on paracetamol-induced toxicity in rats

Serum levels (Mean ± S.E)				
Enzymes and biochemicals	0hour	24hour	48hour	72hour
ALT (U/L)	17.00 ± 00	41.00 ± 0.50*	51.00 ± 0.50*	100.60 ± 0.30*
AST (U/L)	11.00 ± 0.57	81.00 ± 0.50	84.00 ± 0.50*	100.00 ± 0.57*
LDH (U/L)	232 ± 1.52	204.0 ± 2.08*	290.30 ± 0.80*	298.00 ± 0.88*
ALP (U/L)	71.00 ± 0.5	69.60 ± 0.88	66.60 ± 2.40	65.00 ± 1.45
Urea (mg/dl)	7.2 ± 0.03	7.30 ± 0.08	7.02 ± 0.01	7.00 ± 0.21*
Uric acid (mg/dl)	7.2 ± 0.03	8.50 ± 0.14	8.60 ± 0.08	8.50 ± 0.20*
Creatinine (mg/dl)	1.06 ± 0.06	4.06 ± 0.06	4.00 ± 0.00	4.96 ± 0.02

*P < 0.05, **P < 0.01 significant difference; ALT: Alanine transaminase*; AST:* Aspartate aminotransferase; LDH: Lactate dehydrogenase; ALP: Alkaline phosphatase, n=6

Table 3: Effect of lyophilized juice of *C. reticulata* (250mg/kg) fruit on paracetamol-induced toxicity in rats

Serum levels (Mean ± S.E)				
Enzymes and biochemicals	0hour	24hour	48hour	72hour
ALT (U/L)	17.00 ± 00	41.00 ± 0.50**	44.00 ± 0.50**	46.60 ± 0.30**
AST (U/L)	11.00 ±0.57	81.00 ± 0.50**	84.00 ± 0.50**	51.00 ± 0.57**
LDH (U/L)	232 ± 1.52	201.00 ± 2.08	190.30 ± 0.80	170.00 ± 0.88**
ALP (U/L)	71 .00 ± 0.5	69.60 ± 0.88	66.60 ± 2.40	65.00 ± 1.45*
Urea (mg/dl)	7.2 ± 0.03	6.30 ± 0.08	6.02 ± 0.01	5.00 ±0.21**
Uric acid (mg/dl)	7.2 ± 0.03	4.50 ± 0.14	3.60 ± 0.08	3.50 ± 0.20*
Creatinine (mg/dl)	1.06 ± 0.06	3.00 ± 0.06	3.00 ± 0.00	3.00 ± 0.02*

*P < 0.05, **P < 0.01 significant difference; ALT: Alanine transaminase*; AST:* Aspartate aminotransferase; LDH: Lactate dehydrogenase; ALP: Alkaline phosphatase, n=6

Table 4: Effect of lyophilized juice of *C. reticulata* (500mg/kg) fruit on paracetamol-induced toxicity in rats

Serum levels (Mean ± S.E)				
Enzymes and biochemical	0hour	24hour	48hour	72hour
ALT (U/L)	17.9 0 ± 0.49	15.50 ± 0.20**	13.96 ± 0.03**	11.30 ± 0.66**
AST (U/L)	20.30 ± 0.33	17.70 ± 0.30	14.30 ± 1.20*	8.13 ± 0.24**
LDH (U/L)	228.60 ± 2.40	184.00 ± 3.05**	172.30 ± 1.40**	122.00 ± 1.15**
ALP (U/L)	82.30 ±1.45	70.30 ± 0.88*	56.00 ± 3.40**	45.00 ± 2.00**
Urea (mg/dl)	9.00 ± 0.03	8.20 ± 0.06*	6.30 ± 0.08*	2.30 ± 0.08**
Uric acid (mg/dl)	4.50 ± 0.05	4.00 ± 0.06	3.70 ± 0.12*	2.60 ± 0.08**
Creatinine (mg/dl)	1.06 ± 0.06	1.04 ± 0.03	1.04 ± 0.02	0.47 ± 0.17*

*P < 0.05, **P < 0.01 significant difference; ALT: Alanine transaminase*; AST:* Aspartate aminotransferase; LDH: Lactate dehydrogenase; ALP: Alkaline phosphatase, n=6

Table 5: Hepato and nephro-protective effect of lyophilized juice of *C. reticulata* (250mg/kg) fruit on paracetamol-induced toxicity in rats

Serum levels (Mean ± S.E)				
Enzymes and biochemicals	0 hour	24 hour	48 hour	72 hour
ALT (U/L)	21.00 ± 0.57	18.6 ± 0.33	17.30 ± 0.33**	12.90 ± 0.52**
AST (U/L)	18.93 ± 0.52	17.50 ± 0.28	16.40 ± 0.75	14.66 ± 182**
LDH (U/L)	218.30± 4.40	201.60 ± 1.60*	188.00 ± 1.52*	182.00 ± 1.73**
ALP (U/L)	104.33 ± 1.45	96.60 ± 0.88	88.00 ± 3.21*	80.33 ± 0.88**
Urea (mg/dl)	4.20 ±0.05	4.03 ± 0.03	3.36 ± 0.08*	3.20 ± 0.08**
Uric acid (mg/dl)	6.56 ± 0.12	6.13 ± 0.13	5.20 ± 0.1**	4.16 ± 0.44**
Creatinine (mg/dl)	1.16 ± 0.06	1.16 ± 0.03	1.10 ± 0.05	0.97 ± 0.02*

*P < 0.05, **P < 0.01 significant difference; ALT: Alanine transaminase; AST*:* Aspartate aminotransferase; LDH: Lactate dehydrogenase; ALP: Alkaline phosphatase, n=6

Table 6: Hepato-and nephro-protective effect of lyophilized juice of *C. reticulata* (500mg/kg) fruit on Paracetamol-induced toxicity in rats

Serum levels (Mean ± S.E)				
Enzymes and biochemicals	0 hour	24 hour	48 hour	72 hour
ALT (U/L)	16.13 ± 0.40	16.83 ± 0.16	18.33 ± 0.33*	12.80 ± 0.20**
AST (U/L)	13.06 ± 0.43	11.66 ± 0.33*	11.66 ± 1.20*	10.60 ± 0.41**
LDH (U/L)	108.63 ± 0.68	110.66 ± 2.96	111.33 ± 5.69	92.00 ± 5.77**
ALP (U/L)	73.66 ± 4.33	72.33 ± 3.17	63.0 ± 1.15*	56.00 ± 0.01**
Urea (mg/dl)	7.96 ± 0.39	7.84 ± 0.32	6.50 ± 0.28	4.98 ± 0.24**
Uric acid (mg/dl)	8.20 ± 0.20	7.93 ± 0.21	6.73 ± 0.37*	3.36 ± 0.29**
Creatinine (mg/dl)	1.23 ± 0.03	1.10 ± 0.05	1.10 ± 0.00	0.50 ± 0.00**

*P < 0.05, **P < 0.01 significant difference; ALT: Alanine transaminase*; AST:* Aspartate aminotransferase; LDH: Lactate dehydrogenase; ALP: Alkaline phosphatase, n=6

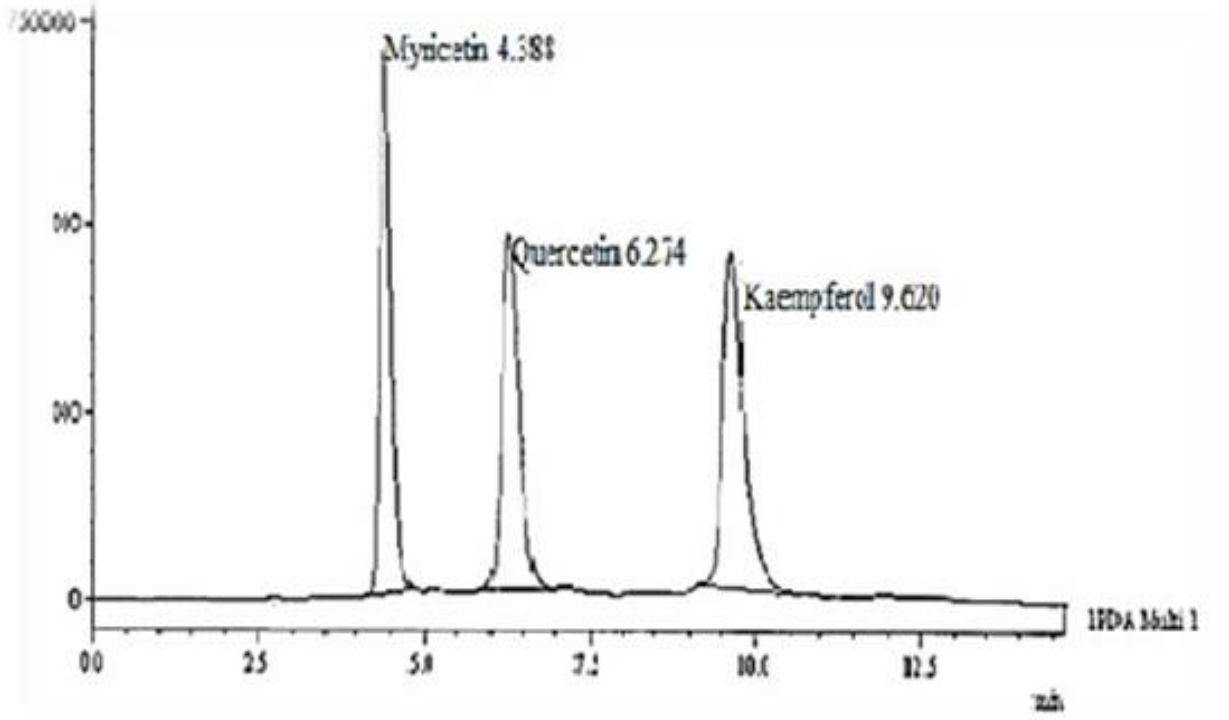

Fig. 1: HPLC chromatograms of flavonoids standards

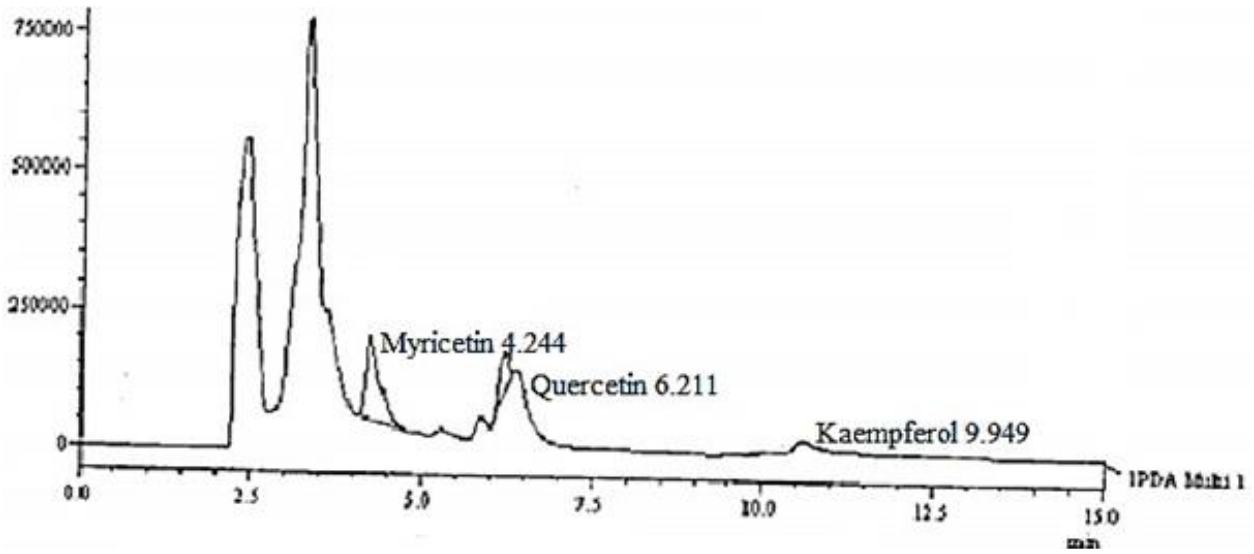

Fig. 2: HPLC chromatogram of lyophilized juice of *C. reticulata* fruit

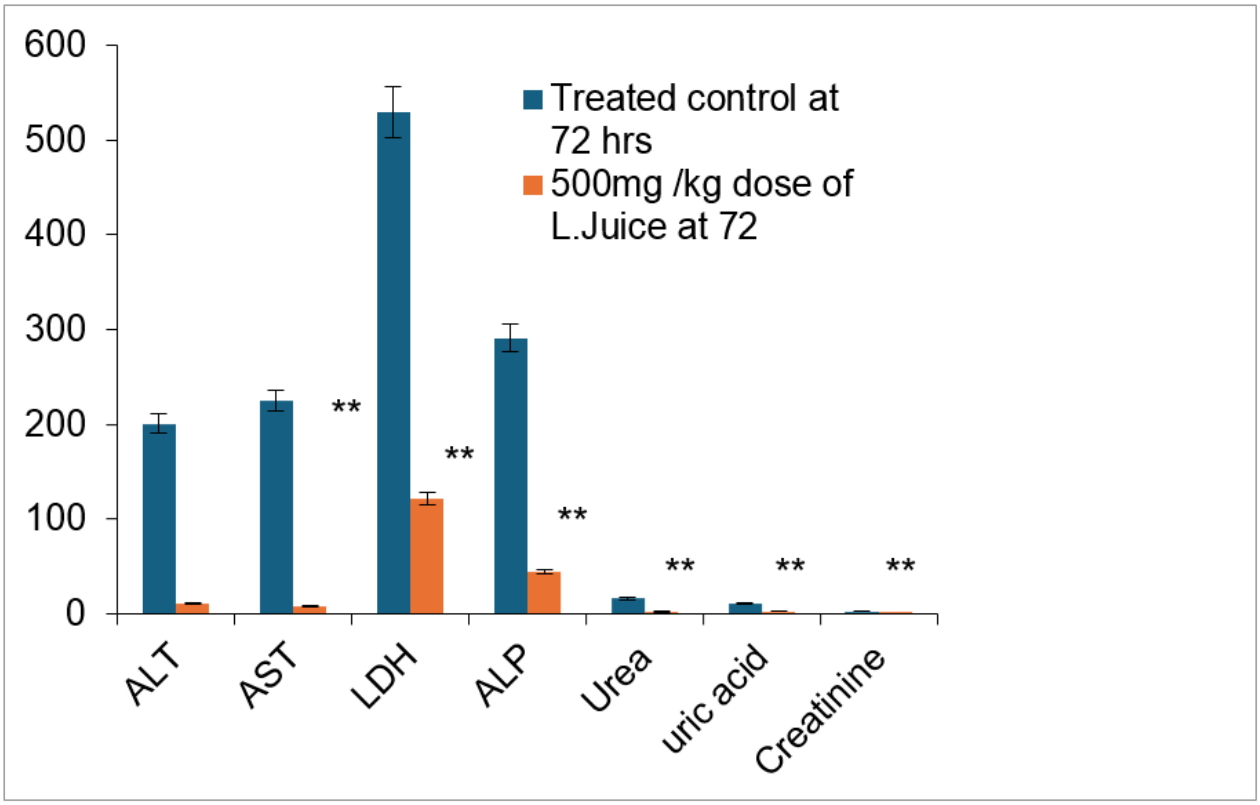

Fig. 3: Comparative effect of lyophilized juice of *C. reticulata* (500mg/kg) fruit on serum biomarkers with treated control at 72 hours.*P<0.05, **P<0.01 significant difference ALT: Alanine transaminase*; AST:* Aspartate aminotransferase; LDH: Lactate dehydrogenase; ALP: Alkaline phosphatase,

The antioxidant and free radical scavenging activities of most plant extracts were mainly due to the presence of flavonoids (Hussen and Endalew, 2023). Similar results were reported earlier that the pharmacological activities i.e. anti-inflammatory, analgesic, antipyretic and astringent activities of plants were due to the presence of flavonoids and tannins (Prasathkumar *et al.*, 2021).

The curative, hepato- and nephro-protective effects of lyophilized juice of *C. reticulata* were studied on paracetamol-induced toxicity in rats. Serum ALT, AST, LDH and ALP, Urea, Uric Acid and Creatinine levels were measured. Lyophilized juice of *C. reticulata* at the doses 150, 250 and 500mg/kg was administered orally to different groups of Paracetamol-treated rats. It was

previously reported that a placebo had no significant effect on serum biochemical parameters; so no placebo reading was taken in the present study. The effect of lyophilized juice of *C. reticulata* compared with zero-hour reading and treated controls (Ali *et al.*, 2018).

Paracetamol-induced liver injury is commonly used as a model for the investigation of the efficacy of hepatoprotective drugs. Seventy-two hours after treatment with Paracetamol (75mg/kg i.p) the ALT, AST, LDH, ALP urea, uric acid, and creatinine levels in the serum increased remarkably. This is because toxic doses of Paracetamol destroy the cellular defense system in hepatic tissues (Saidurrahman *et al.*, 2022). The degree of destruction can be assessed by measuring the metabolism of sulfhydryl compounds, oxygen radicals, and the release of certain enzymes such as ALT, AST, LDH and ALP. Destruction of muscles or liver cells releases liver enzymes with the consequent rise in their values in plasma. In obstructive jaundice and hepatitis, the serum levels of ALT and AST rise to a very high value (300-1500 U/L). Elevation in serum ALT is greater than the rise in AST levels. In extrahepatic obstruction and acute hepatitis, the reverse is true in liver cirrhosis intrahepatic neoplasm and hemolytic jaundice (Malakouti *et al.*, 2017). ALT is present in higher concentration in liver cells than AST, which is present in high concentrations in cardiac muscles and liver, intermediate in skeletal muscles and kidneys, and lower concentrations in other tissues (Bailey *et al.*, 2019).

In hepatocellular injury, the serum activity of LDH rises. Enzyme activities are probably increased as a result of intra-peritoneal absorption of enzymes. These enzymes are released by necrosis of the small amount of hepatic tissue remaining distal to the sight of obstruction. Studies of enzymes released after an acute toxic liver injury have shown that cytoplasmic enzymes are released into circulation within a few hours of injury, which is a reflection of cell membrane Injury or permeability change before the onset of Frank necrosis. Damaged cells leak enzymes into circulation and the rise in the serum level of enzymes and isoenzymes produced by myocardial infractions play an important role in the diagnosis of this disease (Ndrepepa, 2020). The enzymes most commonly measured are creatinine Kinase and lactate dehydrogenase. Lactate dehydrogenase (LDH) is less specific than AST and ALT as a marker of hepatocyte injury. However, it is noteworthy that LDH is disproportionately elevated after an ischemic liver injury. Elevation of serum LDH is also observed in myocardial infarction, pulmonary infarction, hepatic diseases, acute renal infarction and chronic renal disease, hepatitis, and cancer (Klein *et al.*, 2020).

Serum alkaline phosphate activity increases in obstructive jaundice, the values also rise in acute or chronic hepatocellular disease but the increase is greater in former cases. Elevation in serum alkaline phosphate is also associated with pregnancy and bone diseases. High levels are seen with lesions of the liver such as carcinoma, amoebic abscess, amyloidosis and granulomatous lesions, sarcoidosis and tuberculosis of the liver (Siddique and Kowdley, 2012). Paracetamol causes liver damage and nephrotoxicity when taken in over dosage.

Serum urea level rises with impaired renal function. Serum uric acid elevation was observed in gout, renal disease, toxemia of pregnancy, resolving pneumonia, and after irradiation of x-ray-sensitive carcinomas**.** Increased serum creatinine is an indication of acute or chronic damage to the glomerulus of the kidneys (Tesfa *et al.*, 2022).

This was observed that 500 mg/kg lyophilized juice of *C. reticulata* in paracetamol-treated rats decreased the activity of serum enzymes (ALT, AST, LDH, ALP) and serum biochemical (urea, uric acid, creatinine) level more effectively. Comparison of the treated control with the groups taken lyophilized juice of *C. reticulata* fruit in Paracetamol-treated groups showed a significant reduction in the tendency of Paracetamol to raise the activity of serum ALT AST, LDH, ALP and serum biochemical i.e urea, uric acid, creatinine level (table 3 and 4).

Serum enzymes and biochemicals that were increased significantly ($P>0.05$) by paracetamol become normal or lowered after treatment with lyophilized juice of *C. reticulata* fruit (table 4). The mechanisms by which the lyophilized juice of *C. reticulata* fruit exhibited anti-hepatotoxic effects were not investigated in this study. However, according to RP-HPLC analysis bioactive flavonoids myricetin, quercetin, and kaempferol present in lyophilized juice of *C. reticulata* fruit may be inhibiting the biotransformation of paracetamol to N-acetyl-p-benzoquinoneimine or might be fruit juice reduced the extent of necrosis of hepatocytes caused by Paracetamol (Gulati *et al.*, 2018). Literature reports suggested the anti-inflammatory action of the lyophilized juice is due to this bioflavonoid reactivity with membrane phospholipids and membrane enzyme alteration which leads to inhibition of the synthesis of platelet-activating factor (PAF), leukotrienes, and prostaglandins, in addition, they have good antioxidant properties. Might be these bioactive flavonoids and polyphenols reverted Paracetamol-induced hepatotoxicity and nephrotoxicity. The observed hepato-and nephroprotective effect (table 5 and6) of lyophilized juice of *C. reticulata* fruit might be due to their ability to suppress the oxidative degradation of DNA in the tissue debris, because it is known that plant extracts inhibited chromium (VI)-induced free radicals, apoptosis, and deoxyribonucleic acid fragmentation (Okail *et al.*, 2024).

A significant decrease ($P<0.05$) in the activity of transaminases (table3,4, 5 and 6, fig 3) was observed which might be due to specific inhibition of enzyme synthesis. Substrate inhibition, or decrease in concentration/synthesis of co-enzyme, total protein count and decrease the activity of pyridoxal phosphate by lyophilized juice (Swamy *et al.*, 2024).

Literature reported that the fruit of *C. reticulata* when co-administered with a chelating agent gives the optimum effect of chelation treatment; might be that lyophilized fruit juice inhibited the drug metabolism and eliminated it from the body. Lyophilized fruit juice normalizes the liver enzyme (ALT, AST), bile acids, and immune system markers involved in Inflammation and degeneration**.** This complies with the present work because a significant decline in enzymological and biochemical parameters has been observed with the lyophilized juice of *C. reticulata* fruit (Gupta and Flora, 2006).

Decreased activity of serum LDH may be associated with a good response to lyophilized juice of *C. reticulata* fruit therapy. Studies also claimed that Serum ALP activity is also decreased by hypolipidemic agents (Firdous *et al.*, 2021). Phytochemical analysis showed that lyophilized juice of *C. reticulata* fruit is a rich source of unsaturated fatty acids, polyphenols and flavonoids, which are known to have significant anti-atherogenic, cardioprotective and lipid-lowering activity (Otręba *et al.*, 2020). Malnutrition and anemia tend to lower the serum alkaline phosphatase activity (Johnson *et al.*, 2015). Previous literature reported that pretreatment of rats with plant extracts prevented the Paracetamol-induced rise in serum enzyme alkaline phosphatase and transaminase. Similar results were obtained with pretreatment with lyophilized juice of *C. reticulata* fruit. Besides lowering liver enzymes lyophilized juice of *C. reticulata* fruit significantly decreased ($P <0.05$) serum biochemicals i.e. urea, uric acid, and creatinine, which are markers of renal functions.

Usually, serum uric acid levels decrease after administration of the drugs that block the reabsorption of urates from renal tubules or block the step in the formation of uric acid or by defective tubular absorption of uric acid. A decrease in uric acid, urea, and creatinine levels also indicates that kidney function becomes normalized after treatment with lyophilized juice of *C. reticulata* fruit due to its antioxidant compounds (Tesfa *et al.*, 2022), which react with free radicals, and may reduce glomerular damage during nephrosis by protecting LDL particles from oxidation (Duduku *et al.*, 2007). Significant decline was observed in hepatic enzymes and other serum biochemicals at the dose of 500mg/kg lyophilized juice of *C. reticulata*, which not only recovered the liver from paracetamol-induced toxicity but also improved renal function.

In the present study lyophilized juice of *C. reticulata* at the dose of 500mg/kg has curative effects on functioning of liver and kidney, when compared with treated control (fig 3). This effect was maximum at 72hrs of post paracetamol-induced toxicity in .Moreover, current study results are in accordance with findings of Talluri *et al.* (2018) who found positive effect of different plant extracts in reversing the toxic hepatic and nephro-cellular injury after 72hrs. Lyophilized juice of *C. reticulata* fruit is also considered useful in diabetic patients who often have raised ALT levels (Chariyakornkul *et al.*, 2022).

CONCLUSION

In conclusion, lyophilized juice of *C. reticulata* at the dose of 500mg/kg has curative effects on functioning of liver and kidney in paracetamol-induced toxicity in rats. This effect was maximum at 72hrs of post paracetamol-induced toxicity. The lyophilized juice of *C. reticulata* fruit is effective and hepato- and nephro-protective because it efficiently blocks the toxic effect of Paracetamol and prevents hepatic and renal damage. The lyophilized juice of *C. reticulata* fruit harbors various phytochemicals such as flavonoids and polyphenols. The alleviation of paracetamol toxicity might be due to the presence of flavonoids and polyphenols in fruit. The results of *in-vivo* experimentation validated that 500 mg/kg lyophilized juice of *C. reticulata* effectively reduced the levels of serum enzymes ALT, AST, LDH, and ALP and biochemical markers (urea, uric acid, and creatinine) in paracetamol-treated rats However, further investigation, isolation and characterization of these constituents are imperative. These efforts may lead to the identification of potential hepato and nephroprotective agents derived from *C. reticulata*, thereby contributing to the development of effective treatments for drug-induced toxicity.

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