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Original article

Isolation of biomedically important bioactive compounds from *Debregeasia salicifolia* with extraordinary antioxidant potential hepato-protectivity

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ABSTRACT

Debregeasia salicifolia is a widely used medicinal plant that has been used traditionally to cope up different ailments but received little research. So, we have investigated the hepatoprotective and antioxidant properties and also performed chemical profiling by using GC–MS, HPLC and FT-IR techniques. Their analysis revealed the presence of flavonoids, phenols and fatty acids. Albino mice were used for conducting hepatoprotective assay using CCl_4 with three methanol and chloroform extract dosages of (100, 200, and 300 mg/kg b.w.) along with silymarin serving as a positive control. Blood was tested for liver markers to see if they had any hepatoprotective effects. Furthermore, to check healing ability in mice, tests were done by analyzing liver biochemical markers, antioxidant enzymes and direct bilirubins. The high dose of DSM (300 mg/kg b.w) had the most potent hepatoprotective effects on CCl4-induced abnormalities. The in vitro antioxidant, as well as the high quantities of flavonoids in the extracts, coincided well with the in vivo hepatoprotective benefits.

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1. Introduction

Debregeasia salicifolia is a dioecious shrub or small tree, found in Pakistan (Punjab, NWFP, and Kashmir), India, Afghanistan, and Tropical Africa (Ahmad et al., 2013). It belongs to the Urticaceae family and illustrate various therapeutic benefits such as tumor, diarrhea, urinary diseases and skin rashes that are contributed by bio-compounds such as oleic acid, ursolic acid, phenols, triterpenes, lupeol, tormentic acid, pomolic acid, and stigmasterol (Nisa et al., 2011; Zulqarnain et al., 2015).

Carbon tetrachloride (CCl4) is a common hepatotoxin that is used to induce hepatic cytotoxicity because it is metabolized in the liver and releases free radicals, causing lipid peroxidation and hepatocyte necrosis. A single CCl4 exposure can cause severe necrosis and steatosis, which can mimic the signs of acute viral hepatitis (Gul et al., 2020; He et al., 2019). In a mouse model, oxidative stress is a result of chemically induced hepatotoxicity by employing CCl4 (Mahmoodzadeh et al., 2017). CCl4 produces many reactive oxygen species and cytochrome P450 causes liver damage. The liver is a significant organ that plays a critical part in the body's xenobiotic disposal. The hepatoprotective function of natural herbal derived medications must be investigated further, especially when synthetic medications are rarely employed as efficient hepatoprotectives. Due to immense role of antioxidants, natural products have been widely used in the treatment of numerous diseases to detoxify xenobiotics, and effective against infections (Liu et al., 2012; Pareek et al., 2013).

Medicinal plants rich in flavonoids, phenols, and other antioxidant compounds aids in the treatment of diseases. Alzheimer's disease, atherosclerosis, diabetes, cancer, and rheumatoid arthritis are all caused by free radicals such as hydrogen peroxide and singlet oxygen molecules. The cytotoxic, antitumor, anti-hemolytic, and hepatoprotective activities of medicinal plants have become a very reliable and cost-effective source and suggested new drug development ideas for pharmaceutical companies (Letsyo et al., 2017; Russo et al., 2017).

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Because there hasn't been a comprehensive investigation on the protective benefits of DS leaves, the current investigation was done to confirm the potential of DS's therapeutic activity against liver and other organ damage. The primary goal of this research was to see if *Debregeasia salicifolia* Methanol (DSM) and Chloroform (DSC) extracts might protect mice from CCL4-induced toxicity. The goal of this study was to detect phytochemicals, as well as antioxidant and active compounds were identified by HPLC, GCMS, and FT-IR.

2. Methodology

2.1. Sample collection and preparation

D. Salicifolia leaves were obtained from Kotli Sattian (Rawalpindi). Plant specimen (voucher no.138) was submitted at Quaid-i-Azam University's Herbarium of Plant Sciences in Islamabad. Plants were dried in the oven at 60° C then crushed to a powder, and sieved. Using a Soxhlet, 100 g sample was extracted with methanol, and chloroform.

2.2. Estimation of secondary metabolites

Folin-Ciocalteu reagent was used to determine the total phenolic content and a colorimetric test was used to estimate the total flavonoid concentration of the extracts (Abbasi et al., 2015). The grams of QE (Quercetin equivalents) or gallic acid per 500 g/dry weight were used to get the results.

2.3. Antioxidant activity

The ability of DSM and DSC to scavenge free radicals was examined utilizing the DPPH assay to evaluate antioxidant activity by using protocol (Nagai et al., 2005). The deoxyribose technique was used to perform the hydroxyl radical assay (Hilgemann et al., 2010). A UV–vis spectrophotometer measured the absorbance of the working mixture at 520 nm. DSM and DSC were tested for their ABTS (2,2-azinobis[3-ethylbenzothiazoline-6-sulfonate]) free radical scavenging activity (Dudonne et al., 2009). The IC50 value is 50% concentration at which free radicals are quenched by 50.

2.4. High-performance liquid chromatography

A Shimadzu HPLC system (Tokyo, Japan) was used to analyze samples, which included a C18 column and UV/visible detector. A gradient of acetonitrile and 0.1 percent phosphoric acid was used to elute the chemicals (36:64). For all samples, the injection volume was 2 mL at 1 mL/min flow rate, flavonoids were measured at 280 nm and 285 nm with quercetin as a standard.

2.5. Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry Shimadzu is equipped with capillary column, split injection at 250 °C with helium carrier gas. Temperature program for the column oven set at 4 °C/min to 150 °C. The injector temperature was set at 275 °C, with N2 as the carrier gas (1.0 mL/min), a 0.2-L injection volume, and a 50:1 split ratio. Compounds were discovered using the NIST library's spectral data source. By comparing the peak area of each compound to the total peak area, the percentage amount of each compound was computed.

2.6. FT-IR

FT-IR (Fourier transform Infrared) spectroscopy was used to evaluate the plant extract for infrared spectrum analysis with a scan range of 400–4000 cm and a resolution of 4 cm^{-1}. The structural characterization of sample components revealed functional groups with chemical bond types (Dhivya, 2017).

2.7. Hepato-protective assay

2.7.1. Animals

Fifty albino mice with 55.2 ± 2.5 g body weight (b.w), were purchased from National Institute of Health, Islamabad and maintained in standard conditions. After treatment of 100–300 mg/kg b.w, different parameters were measured before and after the treatment to determine acute toxicity such as body weight and serum biomarkers (Gul et al., 2017), antioxidant enzymes (Dacie and Lewis, 1991; Misra and Fridovich, 1972) and liver histopathology (Gul et al., 2021). The institution guidelines are followed for the maintenance of animals which are accordance with ARRIVE guidelines.

	Groups	Treatments
1	Normal control group	Feed + no treatment (21 days)
2	Olive oil group	Feed + 1 mL olive oil (21 days)
3	CCl4 group (-ve Control)	Feed + 1 cc CCl4 /kg b. w via intra-peritoneal means
4	100 mg Methanol group	Feed + CCl4 + methanol extracts 100 mg/kg b. w (Orally)
5	200 mg Methanol group	Feed + CCl4 + methanol extracts 200 mg/kg b. w (orally)
6	300 mg Methanol group	Feed + CCl4 + methanol extract 300 mg/kg b. w
7	100 mg Chloroform group	Feed + CCl4 + chloroform extract 100 mg/kg b. w
8	200 mg Chloroform group	Feed + CCl4 + chloroform extract 200 mg/kg b. w
9	300 mg Chloroform group	Feed + CCl4 + chloroform extract 300 mg/kg b. w
10	Silymarin group	Feed + CCl4 + 100 mg/kg b. w standard drug Silymarin (orally)

2.7.2. Statistical analysis

All the data was statistically analyzed by prism pad-7 software after taking in replicates. The obtained results were represented in mean and standard deviation (mean ± SD) at $p < 0.05$ significance level.

3. Results

The total phenolic content of Debregeasia salicifolia was 224.98 mg/g dry weight (d.w) in methanol extract and 1465.1 mg/g (d.w) in chloroform extract. In methanol, total flavonoid content was 75.12.8 mg/g (d.w), while in chloroform extract; total flavonoid content was 68.4 mg/g (d.w). These results, however are significantly different (p 0.05). DS extracts were tested for antioxidant activity against a variety of different antioxidants assays. In the DPPH experiment, the lowest (inhibition concentra-

Table 1
IC50 value of *Debregeasia salicifolia* leaves.

	ABTS radical cation decolorisation Assay	DPPH free radical scavenging Assay	Hydroxyl radical scavenging Assay
DSM	137.5 ± 6.2	28.9 ± 4	37.4 ± 1
DSC	150.7 ± 1.2	50.6 ± 1.5	52.8 ± 0.5
Ascorbic acid	119 ± 7.9	15.7 ± 3	14.5 ± 0.84
Gallic acid	229 ± 15	24.7 ± 2	16.2 ± 1

All the values were taken in replicates manner with standard deviation whereas results are obtained in μg/ml.

tion) IC_{50} has shown by DS methanol extract at 28.944 g/ml (d.w) (Table 1).

The chromatogram from HPLC has reported the active quercetin peaks at the retention time of 12.5 (Fig. 1) and the details for both methanolic and ethanolic extracts are reported in Table 2.

GC–MS investigation revealed considerable quantities of active compounds with an area % like Oleic acid ($C_{18}H_{34}O_2$) with 42.44%, Ascorbic acid ($C_{38}H_{68}O_8$) with 37.57%, Stearic acid ($C_{18}H_{36}O_2$) with 9.63% and Sebacic ($C_{23}H_{40}O_4$) acid with 0.59% found in *Debregeasia salicifolia* leaves and spectra was presented in Fig. 2a.

Based on the peak values in the IR found in DS samples, FTIR spectroscopy was utilized to identify the functional groups. The results revealed the existence of the following functional groups such as alcohols, alkane, aromatic compounds, and amine stretches at the wavelength of 3242.98, 2992.86, 1413.24 and 1635.42 (Fig. 2b).a

At the end of 2nd week, all of the animals were scarified. CCL_4 reduces the weight of mice and decline in liver size was observed. While the weight of body and liver size was greatly enhanced by raising the extract quantity up to 300 mg/kg b.w (Table 3).

Biochemical markers were reduced as toxicity increased which is induced by CCL_4 (Table 4). DSM extracts at 300 mg/kg b. w increased the defensive effect and normalize the elevated parameters such as ALT 59.6 ± 11 (U/L), AST 75.7 ± 7.2 (U/L), ALP 183.9 ± 58 (U/L) and total bilirubin 0.7 ± 0.09 (g/dL).

Table 2
Quercetin estimation from *Debregeasia salicifolia* extracts.

Samples	Area	Quantity	Retention time
Debregeasia salicifolia Methanol	1,260,931	42.20455	12.5
Debregeasia salicifolia Chloroform	146,963	3.681606	12.5

Results presented that various levels of antioxidant enzymes found in different groups (Table 5). Both extracts DSM and DSC showed protective effect on normalizing the enzymes levels.

Histopathology exposed the changes in cellular organization was markedly reduced after administration of dose and found no fibrosis in *Debregeasia salicifolia* methanol and chloroform extract (Fig. 3).

4. Discussion

Currently, leaves extract of *Debregeasia salicifolia* were investigated for their phytochemical and antioxidant potential. Findings showed that DSM contains active phenolic and flavonoid content. A considerable amount of total phenolic compounds and total flavonoids were found in the preliminary screening of secondary metabolites that act as radical scavengers, reducing agents, and hydrogen donors (Eromosele and Eromosele, 2002). In recent study excellent results were observed against DPPH, ABTS and hydroxyl radical assay when evaluated with standard drugs (gallic and ascorbic acid). The presence of high quantity of phytochemicals such as phenolics indicates the reducing property and greater antioxidant potential (Gul et al., 2020). Previous findings reported the active participation of DS against oxidative stress in in-vitro antioxidant studies (Hartmann and Ober, 2000). In DS, the isoflavonoids component "Quercetin" was measured by HPLC. Quercetin hinders the proliferation of a variety of cancer cell lines and has anti-proliferative properties that are mediated via estradiol. It has a link to the alkaline phosphatase enzyme in the liver (Hassanpour et al., 2011).

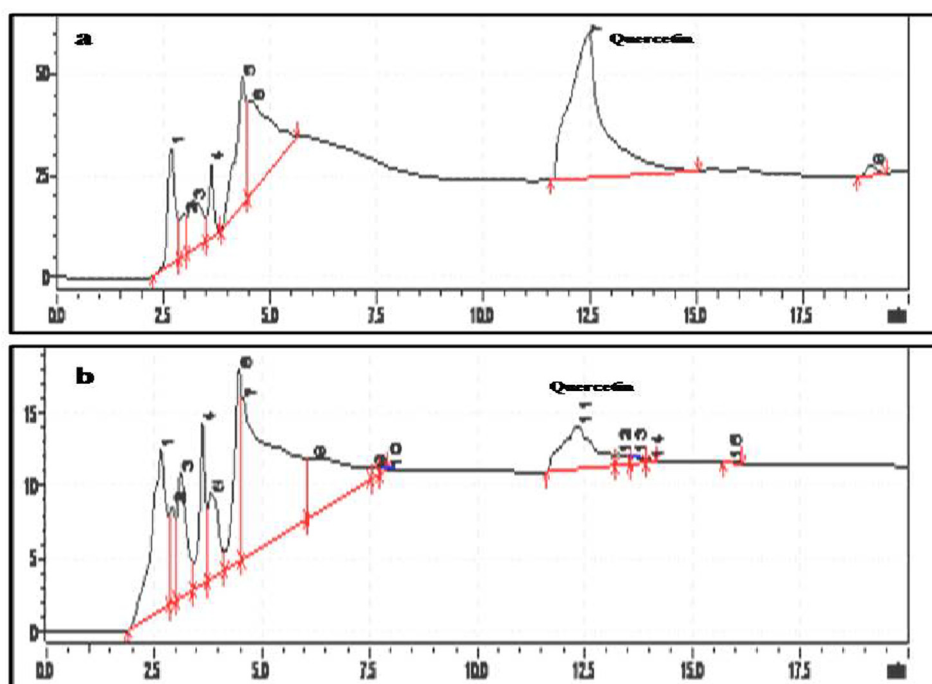

Fig. 1. HPLC chromatogram of (a) Methanol and (b) chloroform extract of *Debregeasia salicifolia.*

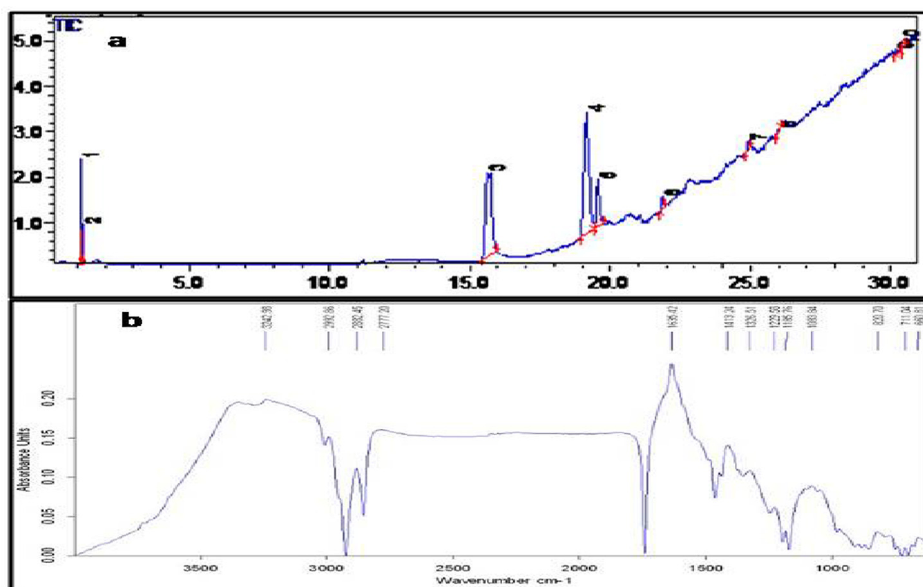

Fig. 2. (a) GC–MS and (b) FTIR spectrums of *Debregeasia salicifolia* leaves.

Table 3
Effect of *Debregeasia salicifolia* leaves on weight of mice.

Treatments	Weight of Liver (g)	Increase (%) in body weight
Normal	5.70 ± 0.41	16 ± 2.9
Olive oil Control	5.82 ± 0.94	20 ± 3.10
CCL_4	3.96 ± 1.18	7.41 ± 2.05
Silymarine + CCL_4	6.02 ± 0.749	22.27 ± 0.09
DSM (100 mg/kg) + CCL_4	4.37 ± 0.40	12.8 ± 2.01
DS M (200 mg/kg) + CCL_4	5.402 ± 0.97	17.12 ± 0.95
DSM (300 mg/kg) + CCL_4	6.38 ± 0.711	25.11 ± 1.83
DSC (100 mg/kg) + CCL_4	4.13 ± 1.22	12.3 ± 1.73
DSC (200 mg/kg) + CCL_4	5.088 ± 0.65	16.43 ± 0.72
DSC (300 mg/kg) + CCL_4	6.17 ± 1.14	23.06 ± 2.42

Data collected in triplicate manners was analyzed with standard deviation.

Oleic acid (Octadecenoic acid), ascorbic acid, stearic acid, glycidol stearate, and sebacic acid were dominating among the chemical substances identified by GCMS in DS extracts. Diet enriched in fatty acids (oleic, linoleic, and linolenic acids) used against inflammation, hypertension, cardiovascular disease, the immunological system, and platelet aggregation (Pantelić et al., 2016; Seniutkin et al., 2018). FT-IR spectroscopy revealed the alcohols, phenol stretch, aromatic compounds and amine stretches in DS. Phenols are bioactive compounds that have been used to make various drugs due to its antioxidant, anticancer and anti-inflammatory potential (Rincón et al., 1999).

In the current study, CCl4 caused lipid peroxidation by interfering with cellular membranes and resulted in damage to the normal integrity of liver mitochondria. It also increased serum transaminases, reduced enzymatic antioxidant defense, and become major source for lipid peroxidation (Agbaje et al., 2009; Kumar et al., 2009). Various plants have been associated to antioxidants and implicated in restoring liver functioning and curing liver damage (Khan et al., 2017). DS extracts significantly improved the activity of serum markers and antioxidant enzymes while also restored cellular integrity in the liver. CCL4 toxicity flattens sub cellular structures (plasma membrane, mitochondria, and endoplasmic reticulum) and increases enzyme activity, and modifies cell membrane permeability, all of which contribute to liver structural damage due to lipid peroxidation (Sreelatha et al., 2009; Jiang et al., 2012). Hepatic damage was revealed to be caused by eminent changes in serum markers and serum enzymes in our study. The levels of hepatic enzymes were measured to estimate the severity of damage tissue (Arun and Asha, 2008).

Biochemical indicators (serum markers) and antioxidant enzymes were modified by carbon tetra chloride and then recovered by treatment with 300 mg/kg DS extracts and the standard drug silymarin. Tissue necrosis, decreased liver capacity and degenerative changes in hepatic cell have all been associated to

Table 4
Analysis of liver biochemical markers.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Direct bilirubin (g/dL)
Normal control	38 ± 0.83	80.2 ± 4.1	110 ± 9.2	0.2 ± 0.001
Olive oil control	40 ± 5.2	63 ± 2	175 ± 20	0.32 ± 0.06
CCl_4 Control	150 ± 9	177.8 ± 8.6	394 ± 15	1.91 ± 0.08
Silymarine drug	66.4 ± 7.2	76.1 ± 12	185 ± 31	0.53 ± 0.09
DSM 100 mg + CCl4	73.4 ± 5.4	108.5 ± 13	331 ± 45	0.84 ± 0.42
DSM 200 mg + CCl4	62.2 ± 8.9	96.1 ± 8	234 ± 7.2	0.79 ± 0.56
DSM 300 mg + CCl4	59.6 ± 11	75.7 ± 7.2	183.9 ± 58	0.7 ± 0.09
DSC 100 mg + CCl4	75.5 ± 9.0	126.7 ± 10	332.5 ± 35	0.94 ± 0.04
DSC 200 mg + CCl4	72.2 ± 14.3	95.5 ± 13.4	253.3 ± 30	0.89 ± 0.1
DSC 300 mg + CCl4	51.5 ± 0.99	93.7 ± 6.5	204.7 ± 7.7	0.81 ± 0.09

Table 5
Estimation of protective effect of DS on antioxidant enzymes.

Groups	CAT (m mol/min/mg protein)	SOD (U SOD/mg protein)	GPx (μ mol/min/mg protein)
Normal control	8.2 ± 2	10.15 ± 1.4	32.10 ± 3
Olive oil	7.9 ± 0.5	10.23 ± 2	34.7 ± 4.5
CCL4 control	3.75 ± 1.9	4.75 ± 0.6	12.83 ± 3
DSM 100 mg + CCL4	7.70 ± 1.2	8.34 ± 2	27.9 ± 1.6
DSM 200 mg + CCL4	8.06 ± 2.5	10.05 ± 3.5	31.14 ± 2.7
DSM 300 mg + CCL4	8.37 ± 1.05	11.22 ± 2	35.02 ± 1.4
DSC 100 mg + CCL4	7.62 ± 2.3	8.10 ± 2.05	26.6 ± 0.8
DSC 200 mg + CCL4	7.88 ± 1.8	9.86 ± 1	30.47 ± 1.5
DSC 300 mg + CCL4	8.17 ± 1.1	10.71 ± 0.8	33.82 ± 0.7
Silymarin + CCl4	8.11 ± 1.2	11.62 ± 0.6	33.7 ± 1.5

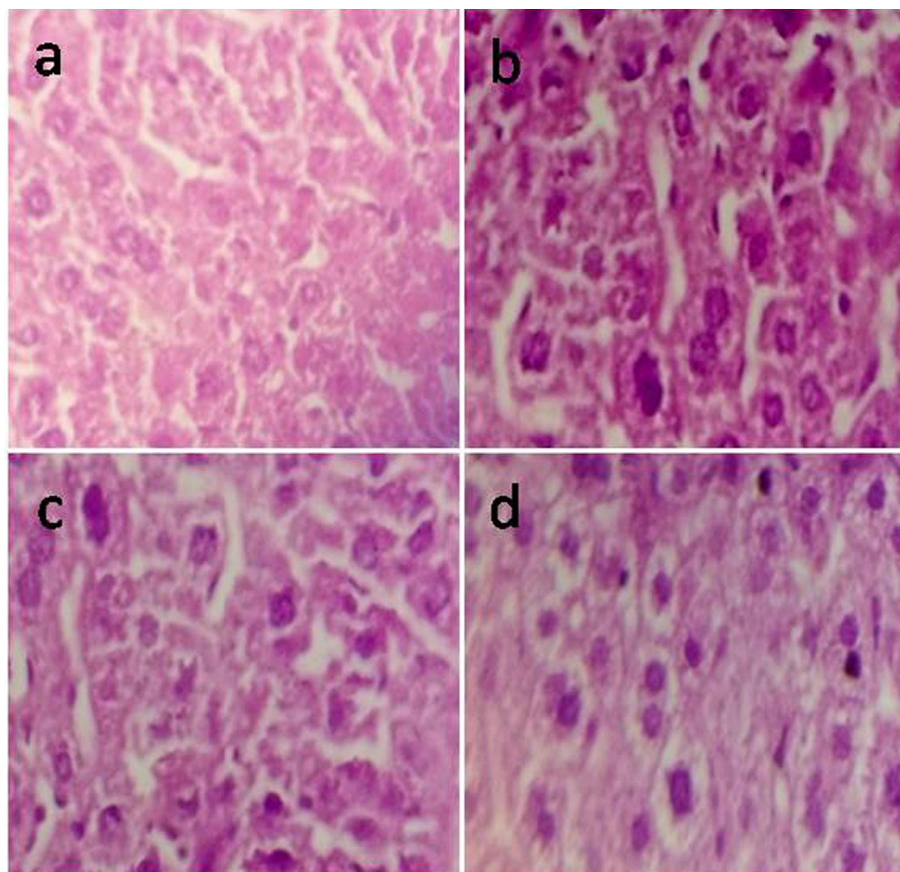

Fig. 3. Histopathological investigation: (a) depicits normal cellular organization where as (b) (c) represents DSM and DSC protective effects and (d) showed slight injurious changes in cellular organization of Liver.

detrimental enzyme alterations (Kumari et al., 2016; Tong et al., 2021). On the other hand, after using plant extracts, the following parameters were restored and reduced toxicity with medicinal herbs (Ullah et al., 2014).

The small changes in animal tissues were seen when treated with the dosage of 100/200 mg/kg of DSM and DSC extracts and there was a small improvement in cellular structure. At high doses 300 mg/kg b.w, DSM extract maintained the structural integrity of the liver and appeared normal with no evident gross morphological or histological alterations. The healing property of DS is due to strong antioxidant property that leads to have potent cancer curing ability (Kumari et al., 2016). These findings have same trend and explain a better resolution of bioactive compounds when compared with results of Tong et al. (2021). Studies revealed that administration of DS aerial parts showed important cytotoxic activity and therapeutic potential reported against different cancer cell lines (Nisa et al., 2011). Important phytochemical compounds present in DS i.e. lupeol, stigmasterol, β-sitosterol and ursolic acid contributed the pharmacological activity in animal model (Ullah et al., 2014).

5. Conclusion

The findings discovered the *Debregeasia salicifolia* pharmacological impact against acute toxicity in mice, signifying its use to treat a variety of ailments, the most common of which are liver illnesses. This plant is rich in antioxidants, flavonoids, phenols and fatty acids. These substances are likely to defend against the effects of carbon tetrachloride on hepatic enzymes. These restorative properties are linked to the plant's traditional use in the treatment of many ailments. This plant could be used to extract potentially ben-

eficial medicines for the treatment of liver and multiple organ injury. To achieve incorporation into curative practice, the active substances and their mode of action, toxicity, efficacy, and molecular mechanisms must all be investigated further.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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