Research Article

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Protective effect of *Helicteres isora*, an efficient candidate on hepatorenal toxicity and management of diabetes in animal models

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Abstract: Diabetes is a metabolic disorder of the carbohydrate pathway, resulting in high blood glucose levels, an increase in thirst as well as urination, problems in vision, weight loss, and high ketone body excretion in urine. Diabetes also affects the endocrine, neurological, and circulatory systems, raising blood pressure and lowering high-density lipoprotein levels with subsequent increases in the low-density lipoprotein level. *Helicteres isora (H. isora)* is a promising medicinal plant having both anti-oxidant and anti-diabetic potentials. Herein, we investigate the protective effect of *H. isora* extracts (crude and fractions) on paracetamol-induced hepatorenal toxicities in animal models. The extracts were initially screened for *in vivo* antidiabetic potential using the alloxan-induced diabetic model. The experimental rats treated with 150 mg/kg b.w. (body weight) dose of Hi-Chl extract decreased the creatinine level to 0.48 ± 0.07 mg/dL, blood urea to 19.64 ± 1.22 mg/dL, and uric acid to 2.23 ± 0.21 mg/dL, indicating the hepatorenal protective functions of the extract. Serum glutamic pyruvic transaminase and alkaline phosphatase were also normalized by the extract to 73.10 ± 4.40 mg/dL and 174.6 ± 2.81 mg/dL, respectively. The Hi-Chl extract exhibited promising anti-diabetic potential with blood glucose normalizing effect from 494.8 ± 2.52 to 159.6 ± 2.67 to 125.6 ± 3.72 mg/dL at doses of 75 and 150 mg/kg, respectively.

Keywords: *Helicteres isora*, hepatotoxicity, nephrotoxicity, diabetes, serum profiling, biochemical analysis

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Abbreviations

H. isora	*Helicteres isora*
Hi-Crd	*Helicteres isora* crude extract
Hi-Chl	*Helicteres isora* chloroform fraction
Hi-Et	*Helicteres isora* ethyl acetate fraction
SGPT	serum glutamic pyruvic transaminase
ALP	alkaline phosphatase
LDL	low-density lipoprotein HDL
HDL	high-density lipoprotein HDL
TGs	triglycerides
mg/dL	milligram per deciliter
mg/kg b.w.	milligram per kilogram body weight
ROS	reactive oxygen species
GCMS	gas chromatography-mass spectrometry
HPLC	high-performance liquid chromatography
LD	lethal dose

1 Introduction

Toxicity in humans is caused by chemical substances or environmental factors resulting in undesirable effects in humans upon exposure, ranging from simple and mild

symptoms to even death depending upon the type of the toxic agent, level, and duration of exposure. There are different routes through which toxic agents enter into human body, such as through oral, inhalation, injection (via insect's bites or needle), and topical (skin absorption). Alloxan is a chemical substance known for its toxic effects on beta cells of pancreas that produces insulin. Alloxan when reaches blood circulation is selectively accumulated by the beta cells of pancreas and produces reactive oxygen species (ROS), involving a series of complex chemical reactions. ROS initiates oxidative stress and damages the beta cells causing impairments in the production of insulin resulting in poor absorption of glucose from the blood, a condition called hyperglycemia. This can also produce toxic effects on the vital organs like kidneys, resulting in damages to nephron, other vital organs like liver, and heart. Diabetes normally results due to irregularities or abnormalities in insulin action or its secretion and has detrimental effects on various organ functions; for example, renal functions, nerve functions, cardiac functions, and vision are badly affected [1]. Diabetes is of two types: insulin dependent diabetes mellitus (IDDM), or type I diabetes, and non-insulin dependent diabetes mellitus (NIDDM), also known as type II diabetes. In IDDM, there is a decrease in the production of insulin due to the destruction of the cells in pancreatic islets of Langerhans [2,3]. NIDDM occurs when the cells lose sensitivity to insulin or when insulin receptors are covered with fat, and obesity is one of the primary factors. The malfunctioning of organs in diabetes is a result of abnormalities in fat, protein, and carbohydrate metabolism resulting in increased level of blood glucose [4]. Numerous signs of diabetes can appear, such as polydipsia (increased thirst, vision problems) and unexplained weight loss. Additionally, in diabetes, more ketone bodies can be observed in the urine [5]. Diabetes can lead to neuropathic conditions, sexual dysfunction, and cardiovascular complications often characterized by elevated blood pressure, high level of low-density lipoprotein (LDL), commonly known as "bad" cholesterol, and decreased level of high-density lipoprotein (HDL), often called "good" cholesterol. This means that the changes brought about by diabetes in the lipid profile increases the risk of heart diseases. Hyperglycemia triggers a specific metabolic pathway involving protein kinase C (PKC), diacylglycerol (DAG), and NADPH oxidase, ultimately resulting in the production of ROS. This pathway has been previously proposed as the "dangerous metabolic route in diabetes" [6].

Medicinal plants have been in use for centuries in various cultures for treating many health complications, including toxicity and diabetes. Medicinal plants possess anti-inflammatory, anti-oxidant, and hepatoprotective potential, which can also promote the removal of toxins from body and protect kidneys and liver from their harmful effects [7–9]. Studies have revealed that medicinal plant extracts have potential effects on the blood glucose level in the management of diabetes [10] and even some stimulate and improve secretion of insulin or inhibit absorption of glucose from the digestive tract offering beneficial effects in the management of diabetes [11].

Helicteres isora Linn, a plant species in genus *Helicteres* (family; malvaceae), has exhibited antidiabetic potential and is considered to be an excellent drug candidate that can effectively normalize the functions of pancreatic islets and regulate insulin activity by virtue of associated antioxidant properties. Its fruit has been found effective in lowering blood glucose level and hence is used widely in Asian communities for therapeutic purposes. The hot water extract of *H. isora* fruits has demonstrated significant anti-hyperglycemic activity, surpassing that of standard drugs, as observed in various experimental models [12]. Our previous study has revealed the safety profile as well as phytochemical analysis of *H. isora,* where the beneficial role of the plant extract in vital organs such as pancreas, heart, liver, and kidneys was explored targeting the related biochemical parameter investigation. The reported study was aided with high-performance liquid chromatography analysis, which confirmed the presence of phytochemicals such as steroids, flavonoids, alkaloids, sugars, and terpenoids, among which quercetin was the most common compound [13]. Herein, we aimed to investigate the hepato-renal protective effects of *H. isora*, and also to determine its antidiabetic potential in an animal model with aided instrumental analysis of GCMS.

2 Materials and methods

2.1 Chemicals and reagents

Analytical grade chemicals ethyl acetate (141-78-6 Sigma Aldrich), n-hexane (110-54-3 Sigma Aldrich), methanol (67-56-1 Sigma Aldrich), AChE (9000-81-1 Merck), butyryl thiocholine iodide (1866-16-6 Merck), chloroform (67-66-3 Sigma Aldrich), 5,5-dithio-bis-nitrobenzoic acid (69-78-3 Merck), BuChE (9001-08-5 Merck), Tween-80 (9005-65-6 Merck), and donepezil hydrochloride (120011-70-3 Merck) were procured from the mentioned firms and used without further purifications.

2.2 Plant collection, extraction, and fractionation

The selected plant *Helicteres isora* (local name: Patch Pali) fruits were procured from the local bazaar in Peshawar, Khyber Pakhtunkhwa, and were identified by a taxonomist. The

voucher specimen (Hi/10/22) was deposited in the University of Malakand's herbarium. The fruits weigh 2.5 kg. They were ground and soaked for about 2 weeks at room temperature in methanol (commercial grade, 80%), followed by filtration and concentration using a rotary evaporator to obtain the crude extract (Hi-Crd, 375 g). The Hi-Crd was further subjected to fractionation to obtain the chloroform fraction (Hi-Chl, 52 g) and ethyl acetate (Hi-Et, 41 g), along with hexane, butanol, and aqueous extracts [14].

2.3 Gas chromatography-mass spectrometry (GC-MS) analysis

The chloroform fraction (Hi-Chl) was subjected to GC-MS analysis using gas chromatograph (Agilent USB-393752) equipped with a capillary column with dimensions of 30 m × 0.25 mm. The thickness of the film was 0.25 µm. A mass selective detector Agilent HP-5973 was coupled with the system operating in the electron impact mode with an ionization energy of 70 eV. The analysis was started (1 min at 70°C) and then inclined to 180°C at a rate of 6°C/min; this temperature was maintained for 5 min. Finally, the temperature was raised to 280°C over a 20-min period at a rate of 5°C/min. The temperature of the injector was set to 220°C, and that of the detector was maintained at 290°C. Before injection in the splitless mode, the samples were diluted (1/1,000 in *n*-pentane, v/v) to a volume of 1 µL. The carrier gas used to transport compounds was helium at a flow rate of 1 mL/min. When the eluted fractions left the GC column, they underwent chemical ionization before entering the mass spectrometer. In the mass spectrometer (MS), the mass-to-charge (*m*/*z*) ratios of the ions were used to separate and identify the ions.

2.4 Identification of components

Compounds were identified by their retention time and through comparison with existing data documented in the literature. Furthermore, spectral data from Wiley and NIST libraries were used to analyze fragmentation patterns observed in the mass spectra, serving as an additional tool for identification [15].

2.5 *In vitro* α-glucosidase inhibitory activity

The α-glucosidase activity was determined by mixing α-glucosidase (20 µL, 0.5 unit/mL), in phosphate buffer (0.1 M, pH 6.9) with Hi-Crd and the fractions (10 µL). The mixture was incubated in 96-well plates for 15 min at a temperature of 37°C. The reaction was initiated by the addition of *p*-nitro phenyl-*α*-D-glucopyranoside solution (20 µL, 5 mM) in phosphate buffer (0.1 M, pH 6.9), followed by further incubation for 15 min. Sodium carbonate (0.2 M, 80 µL) was added to stop the reaction and the absorbance was recorded at 405 nm using a microplate reader. A reaction system without samples was used as a positive control. Additionally, a blank without α-glucosidase was included to account for background absorbance [16].

2.6 Experimental animals and ethical approval

Male rats (150–180 g, 6–8 weeks old) were procured from the National Institute of Health Islamabad and kept in the University of Malakand animal house with standard housing conditions of light and dark cycle of 12 h, temperature of 25 ± 2°C, and relative humidity of 55–65%. The Departmental Ethical Committee (Pharm/DEC/16/22) endorsed the study in compliance with the 2008 Animal Bye-Laws. Standard pellet food was given to the animals, and they had full access to water [13].

2.7 Acute toxicity and selection of doses

Rats were divided into test and control groups, with six animals in each group (n = 6). Hi-Crd was administered to the respective test groups at doses ranging from 500 to 5,000 mg/kg b.w. After administration, the animals were closely monitored for 72 h and then observed for a period of 14 days for signs of tremors, diarrhea, salivation, convulsions, lethargy, and sleep disturbances. The animals in the groups were provided free access to water and food. The body weight of animals was monitored on a weekly basis. During these 14 days, animals were observed daily.

2.8 Hepato- and nephroprotective activity

Hepatotoxicity and nephrotoxicity in animals was induced by administering a high dose of paracetamol (300 mg/kg b.w) orally for 7 days. Animals were categorized randomly in groups comprising the normal control group, negative control group, and test groups. The animals in the respective test groups received 150 and 300 mg of Hi-Crd and 75 and 150 mg/kg b.w. of Hi-Chl and Hi-Et, respectively [17,18].

2.9 Induction of diabetes

Diabetes was induced by injecting alloxan (dissolved in citrate buffer) intraperitoneally (i.p.) to the overnight fasted rats at a dose of 150 mg/kg b.w. Following a 72-h period after the alloxan injection, blood was taken from the tail vein and the glucose level in blood was determined using a glucometer (VIVA-CHECK, Active blood glucose meter, Korea). Animals with the fasting blood glucose level higher than 250 mg/dL were considered diabetic [16].

2.9.1 Experimental design for antidiabetic activity

Animals were randomly categorized into various groups with n = 8, including the normal group, diabetic control group (only vehicle was administered), the standard group treated with glibenclamide (0.5 mg/kg), and diabetic groups treated with 150 and 300 mg/kg b.w. of Hi-Crd, 75 and 150 mg/kg Hi-Chl and Hi-Et daily, respectively, for a period of 28 days [16].

2.9.2 Estimation of the blood glucose level and body weight

Blood glucose level was monitored on day 1 of each week, while the weight of animals was also noted [16].

2.10 Estimation of serum profile

After completion of an antidiabetic activity (28 days), animals were anesthetized by administering an i.p. dose of 35 mg/kg pentobarbital sodium to ensure that the animals completely lost consciousness without pain. The blood sample was obtained by cardiac puncture, which were subsequently used to assess total cholesterol (TC), high-density lipoprotein (HDL), low density lipoproteins (LDL), and triglycerides (TG) levels [16].

2.11 Histological studies

The animals, after blood collection, were dissected using OECD guidelines to excise the kidneys, liver, and pancreas, washed in a chilled saline solution (0.9% NaCl), gently dried with filter paper, and the weight of relative organs was determined.

The organs were sectioned by microtome followed by staining with hematoxylin and eosin stain and examined (scale bar 25 µm) under an Optika microscope (B-5101BF, SN 573676 Italy) with 40× magnifications.

2.12 Statistical analysis

Data are expressed as mean ± SEM with n = 8. One-way ANOVA and Dunnett's *post-hoc* test were used to determined statistical significance by using graph pad prism.

3 Results

3.1 GC-MS analysis

The results of GCMS and the most common compounds of *H. isora* are displayed in Figure 1, which shows that phytochemicals are concentrated in the Hi-Chl fraction, and these secondary metabolites in plants are known to have the potential to provide defense and therapeutic properties. If they are continuously incorporated into the diet, they may provide protection against a wide range of diseases [19]. GC-MS analysis (Figure 1) displays the phytochemical constituents of the *H. isora*: aR-turmerone, (E)-atlantone, oleic acid, 6-octadecenoic acid methyl ester, linoleic acid, octadecanoic acid, stigmasterol campesterol, and sitosterol. Octadecanoic acid acts as an anti-cancer agent, induces apoptosis, and also acts as an anti-inflammatory agent [20]. aR-Turmerone exhibits anti-inflammatory, anticonvulsant, anti-metastatic anticancer, and antifungal activities [21]. Linoleic acid has a prominent role in cardiovascular health. Clinical studies have revealed that linoleic acid substitutes saturated fats and results in a decrease in the levels of both LDL and cholesterol. Some studies provide evidence that linoleic acid enhances the sensitivity of insulin and also regulates blood pressure [22]. Oleic acid is an unsaturated omega-9 fatty acid found in both animals and plant sources and acts as a solubilizing and emulsifying agent in aerosol products. Oleic acid hinders the progression of adrenoleukodystrophy, a devastating condition that affects adrenal glands and brain, and it also enhances memory [23]. Campestrol (sterol found in plants) reduces cholesterol levels and also acts as an anti-inflammatory agent [24]. Stigmasterol is also a phytosterol and is extensively assessed in *in vitro, in vivo,* and *in silico* studies for a broad range of pharmacological activities as anticancer agent, anti-osteoarthritis agent, anti-

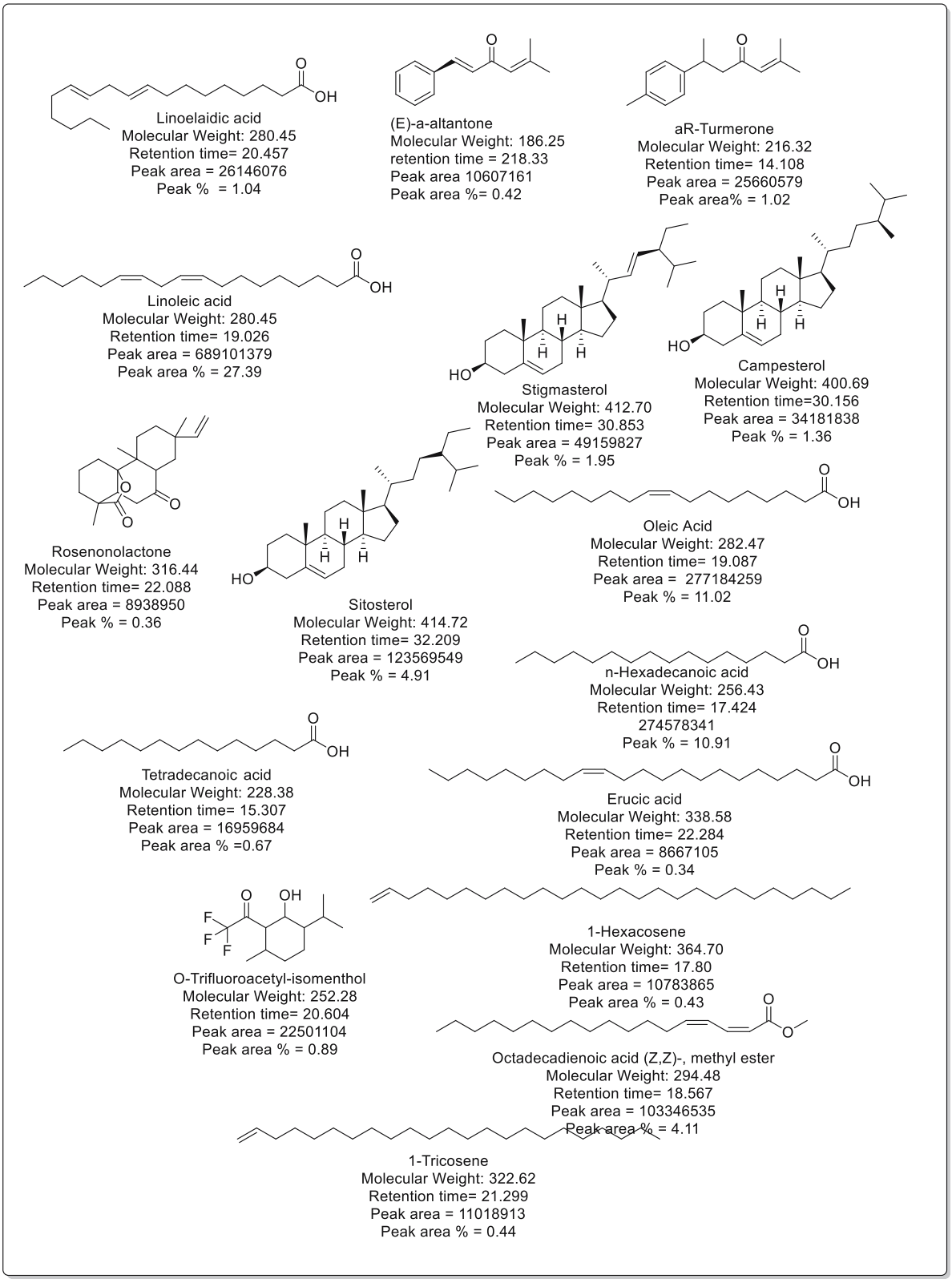

Figure 1: GCMS results and phytochemical constituents of *H. isora.*

inflammatory agent, anti-diabetic agent, immune modulator, anti-parasitic remedy, antifungal agent, antibacterial substance, antioxidant, and neuroprotective agent [15]. Sitosterol has been used to effectively reduce the level of cholesterol, LDL, and also improves symptoms of mild to moderate benign prostatic hypertrophy. Furthermore, beta-sitosterol has been the subject of research for its potential immunomodulatory and anticancer properties. This compound, which is structurally similar to cholesterol, is the most prevalent plant sterol as its structure resembles that of beta-sitosterol and can substitute for cholesterol in the human body [16].

3.2 Acute toxicity and dose selection

Results from acute toxicity reveal that animals treated with Hi-Crd extract tolerated up to 4,000 mg/kg and found to have no adverse effects. When the dose was increased to 5,000 mg/kg, mortality of 60% was observed, and the LD_{50} was found to be 4,472 mg/kg.

3.3 Biochemical analysis for the hepatorenal protective effect

To ensure the protective effect of Hi-Crd and fractions on hepatorenal toxicity, the biochemical analysis of bilirubin, SGPT, and ALP for the liver function test and serum creatinine, blood urea and uric acid for the liver function test were performed.

3.3.1 Liver function tests

The results of the liver function test for assessment of biomarkers, including bilirubin, SGPT and ALP, are presented in Table 1. In the negative control group (paracetamol), elevated level of bilirubin 0.73 ± 0.04 mg/dl was monitored in comparison to the normal control group (0.21 ± 0.04) that was normalized to 0.24 ± 0.04 ($P < 0.001$) when treated with the standard drug, indicating a prominent decline by the standard drug to cure toxicity. When animals were treated with Hi-Crd and fractions, the significant results of reducing the bilirubin level were noted with Hi-Chl administration at a dose of 150 mg/kg (0.26 ± 0.06; $P < 0.001$). The SGPT levels in the standard group, paracetamol control group, and normal group were 73.30 ± 2.44 ($P < 0.001$, n = 8), 118.5 ± 3.96, and 67.10 ± 2.92, respectively. Animals treated with 150 and 300 mg/kg b.w. of Hi-Crd showed a decrease in the SGPT levels of 101.07 ± 2.38 and 88.50 ± 2.10, respectively. Chloroform (Hi-Chl) and ethyl acetate (Hi-Et) fractions at 75 and 150 mg/kg showed 82.3 ± 2.51, 73.10 ± 4.40, 106.1 ± 3.56, and 94.10 ± 2.91, respectively, and were statistically significant.

Paracetamol-induced toxicity also altered the ALP values and elevated the levels in the paracetamol control group to 207.5 ± 3.34 in comparison to the normal control group (157.1 ± 3.09). Treating animals with *H. isora* and fractions cured toxicity induced by paracetamol. Animals administered with 150 and 300 mg/kg Hi-Crd displayed a statistically significant decline in the ALP value (191.6 ± 2.41 and 182.6 ± 2.89, respectively). Animals treated with 75 and 150 mg/kg Hi-Chl and Hi-Et decreased the ALP level to 173.3 ± 2.70, 174.6 ± 2.81 and 193.60 ± 2.81, 188.5 ± 2.70, respectively, and were found significant statistically.

3.3.2 Kidney function tests

The results of the kidney function test are presented in Table 2. Paracetamol increased the creatinine levels almost double to that of the normal control group and were found

Table 1: Biochemical analysis of bilirubin, SGPT, and ALP for liver function tests

Biochemical analysis	Bilirubin (mg/dl)	SGPT (U/l)	ALP
Normal	0.21 ± 0.04	67.10 ± 2.92	157.10 ± 3.09
Paracetamol	0.73 ± 0.03	118.50 ± 3.96	207.50 ± 3.34
Silymarin 40 mg/kg	0.24 ± 0.07***	73.30 ± 2. 44***	169.10 ± 2.86***
Hi-Crd 150	0.35 ± 0.05***	101.07 ± 2.38**	191.60 ± 2.41**
Hi-Crd 300	0.30 ± 0.04***	88.50 ± 2.10***	182.60 ± 2.89***
Hi-Chl 75	0.32 ± 0.07***	82.30 ± 2.51***	173.30 ± 2.07***
Hi-Chl 150	0.26 ± 0.06***	73.10 ± 4.40***	174.60 ± 2.81***
Hi-Et 75	0.36 ± 0.07**	106.10 ± 3.56*	193.60 ± 2.81**
Hi-Et 150	0.32 ± 0.04***	94.10 ± 2.911***	188.50 ± 2.70***

Data are expressed as mean ± SEM with $n = 8$. One-way ANOVA and Dunnett's *post-hoc* test were used to determine statistical significance. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ were considered significant compared to the control group.

to be 0.91 ± 0.08 for the paracetamol control group and 0.43 ± 0.05 for the normal control group, respectively. The level of creatinine in the standard group was 0.45 ± 0.05 ($P < 0.001$, $n = 8$). Animals that received 150 and 300 mg/kg of Hi-Crd showed declined creatinine levels of 0.57 ± 0.06 and 0.51 ± 0.04 ($P < 0.05$ and $P < 0.001$), respectively. Animals treated with 75 and 150 mg/kg Hi-Chl showed creatinine levels of 0.55 ± 0.11 ($P < 0.01$) and 0.48 ± 0.07 ($P < 0.001$), while Hi-Et administration showed creatinine levels of 0.62 ± 0.07 and 0.57 ± 0.08 ($P < 0.05$, $P < 0.01$), respectively. The levels of blood urea in the normal control group and paracetamol control group (toxic group) were 13.60 ± 0.66 and 41.30 ± 2.01, respectively, indicating a threefold increase in the blood urea level to that of normal control ($P < 0.001$, $n = 8$) (paracetamol control group). Administration of Hi-Crd and fractions reverted the blood urea levels. Hi-Crd at the respective doses decreased significantly the blood urea level to 29.30 ± 2.72 and 21.60 ± 2.17. Animals treated with 75 and 150 mg/kg chloroform and ethyl acetate fractions showed a decrease in the urea levels of 23.30 ± 2.10, 19.64 ± 1.22, 31.0 ± 1.57, and 28.33 ± 1.35, respectively. Similar results were produced during the assessment of uric acid level when the Hi-Crd and fractions at respective doses were administered into the paracetamol induced nephrotoxicity model.

3.4 *In vitro* α-glucosidase activity

The inhibitory potential of alpha-glucosidase by *H. isora* is presented in Table 3 with reference to acarbose as a standard. Compared to the standard, maximum inhibition of alpha-glucosidase was produced by the Hi-Chl fraction with an IC_{50} value of 122.63 µg/mL, followed by the Hi-Crd fraction with an IC_{50} value of 140.34 µg/mL. The least inhibition was displayed by the Hi-Et fraction (220.26 µg/mL). The IC_{50} value of the standard acarbose was 12.54 µg/mL. This *in vitro* study suggests the potential of *H. isora* in the management of diabetes.

3.5 Anti-diabetic activity

Figure 2 displays blood glucose levels of normal control, diabetic control, and groups of diabetic rats treated with methanolic extract Hi-Crd, Hi-Chl, and Hi-Et, as well as standard glibenclamide. The blood glucose levels of animals in the normal control group were found to be in the range of 107.1 ± 1.64 to 114.1 ± 2.84 and 110.0 ± 2.12. In the diabetic control group, the blood glucose levels were found to be 481.1 ± 2.05, 486.5 ± 3.87, 494.1 ± 3.48, 498.3 ± 2.57, and 503.10 ± 3.80 mg/dL, respectively. The Hi-Crd administered at 150 and 300 mg/kg on day 14 decreased the blood glucose level to 348.8 ± 3.27 and 333.5 ± 2.17 ($P < 0.001$, $n = 8$), while on day 28 of the study, it was found to be 151.6 ± 3.91 and 119.1 ± 2.01 ($P < 0.001$, $n = 8$), respectively. Diabetic animals treated with the Hi-Chl fraction at a dose of 75 and 150 mg/kg significantly declined the blood glucose level to 159.6 ± 2.67 and 125.6 ± 3.72 ($P < 0.001$, $n = 8$)

Table 3: Effect of *H. isora* extracts on the α-glucosidase enzyme inhibition activity with IC_{50} values

Sample	*α*-Glucosidase inhibitory activity with IC_{50} values(µg/mL)
Acarbose	12.54
Hi-Crd	140.34
Hi-Chl	122.63
Hi-Et	220.26

Table 2: Biochemical analysis of the kidney function test

Biochemical analysis	Serum creatinine (mg/dl)	Blood urea (mg/dl)	Uric acid (mg/dl)
Normal	0.43 ± 0.05	13.60 ± 0.66	1.32 ± 0.21
Paracetamol	0.91 ± 0.08	41.30 ± 2.01	4.54 ± 0.25
Silymarin 40 mg/kg	0.45 ± 0.05***	18.10 ± 1.19***	2.07 ± 0.25***
Hi-Crd 150	0.57 ± 0.06*	29.30 ± 2.72***	3.03 ± 0.3**
Hi-Crd 300	0.51 ± 0.04***	21.60 ± 2.17***	2.71 ± 0.12***
Hi-Chl 75	0.55 ± 0.11**	23.30 ± 2.10***	2.82 ± 0.26***
Hi-Chl 150	0.48 ± 0.07***	19.64 ± 1.22***	2.23 ± 0.21***
Hi-Et 75	0.62 ± 0.07*	31.00 ± 1.57**	3.38 ± 0.24*
Hi-Et 150	0.57 ± 0.08**	28.33 ± 1.35***	3.40 ± 0.10**

Data are expressed as mean ± SEM with $n = 8$. One-way ANOVA and Dunnett's *post-hoc* test were used to determine statistical significance. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ were considered significant compared to the control group.

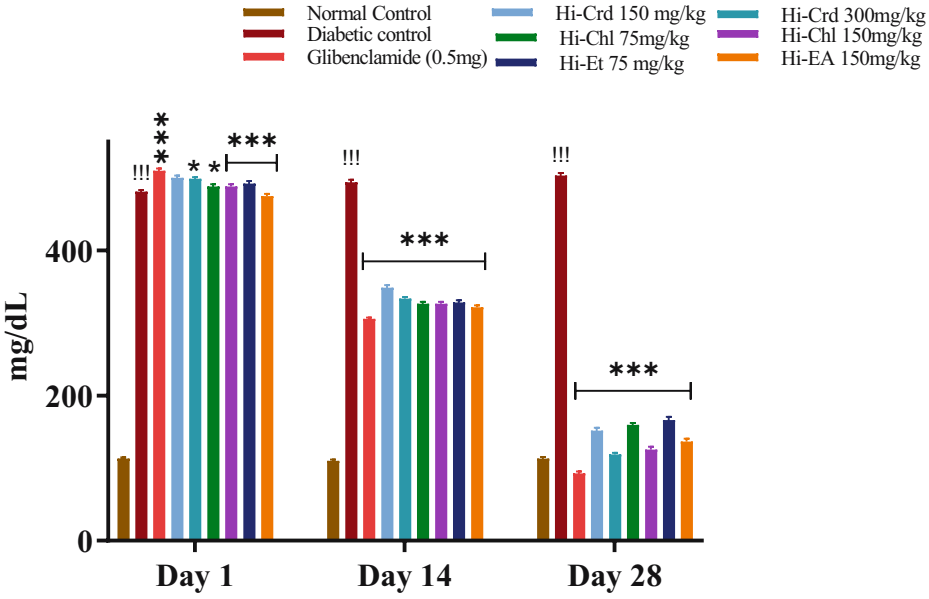

Figure 2: Determination of blood glucose levels. Data are expressed as mean ± SEM with $n = 8$. One-way ANOVA and Dunnett's *post-hoc* test were used to determine statistical significance. !!!$P < 0.001$ was considered significant compared to the control group. Within the diabetic group, differences were noted with significance levels of *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

on day 28. Animals administered with 75 mg/kg b.w of Hi-Et produced a decline in the blood glucose level of 328.5 ± 2.43 and 166.50 ± 3.72 ($P < 0.001$, $n = 8$), respectively, on days 14 and 28. When animals received 150 mg/kg b.w. dose of Hi-Et, the blood glucose levels were 321.8 ± 2.41 and 136.8 ± 3.69 ($P < 0.001$, $n = 8$), respectively, on days 14 and 28. The findings from the study reveal the Hi-Chl of plant displayed promising anti-diabetic effects that caused a gradual decrease in the levels of blood glucose.

3.6 Effect on body and organ weight of diabetic rats

The body weights of animals in the normal control group, diabetic control group, and diabetic animals treated with Hi-Crd, Hi-Chl, and Hi-Et, as well as standard glibenclamide, are shown in Figure 3a and b. Animals in the diabetic control group significantly lost 16.8 g of weight till day 28 of the study The standard glibenclamide, Hi-Crd, and its fractions (Hi-Chl and Hi-Et) produced a significant effect ($P < 0.05$, $P < 0.01$, $P < 0.001$, $n = 8$) by reversing the weight loss during the study period, indicating a modulatory effect. The weight of the pancreas in the normal group was 0.63 ± 0.04 g, 0.48 ± 0.01 g in the diabetic control group, and 0.74 ± 0.04 in the group treated with standard glibenclamide. Groups that received Hi-Crd at 150 and 300 mg/kg b.w. produced a profound effect on the pancreas weight that was found to be 0.86 ± 0.02 and 0.86 ± 0.02 g, respectively. Animals treated with 75 and 150 mg/kg Hi-Chl and Hi-Et elevated the pancreas weight to 0.67 ± 0.03, 0.73 ± 0.0, 0.70 ± 0.02, and 0.77 ± 0.04 g, respectively, in comparison to the diabetic group.

3.7 Effects on lipid profile

The results of the lipid profile are shown in Figure 4a and b, which shows a significant increase in the level of cholesterol compared to the diabetic control group. In the diabetic control group, a significant rise in the cholesterol level was observed and found to be 189.5 ± 4.13 mg/dL ($P < 0.001$, $n = 8$) compared to the normal control group (38.66 ± 1.97 mg/dL). Animals treated with Hi-Crd regulated the cholesterol level to 70.3 ± 3.14 and 67.83 ± 1.53 mg/dL at 150 and 300 mg/kg, respectively, compared to the diabetic group. Animals treated with Hi-Chl and Hi-Et at respective doses produced similar results and reverted the cholesterol level compared to the diabetic group. In diabetic rats, an increase in the level of triglycerides was noticed ($P < 0.05$, $n = 8$) and was found to be 118.00 ± 6.18 compared to the normal control group. Animals treated with 150 mg/kg Hi-Crd altered the HDL levels to 90.16 ± 2.00, and, at a dose of 300 mg/kg, it decreased to 101.50 ± 2.84 mg/dL. Animal treated with 75 and 150 mg/kg Hi-Chl and Hi-Et decreased the triglyceride level to 111.30 ± 3.36, 109.30 ±

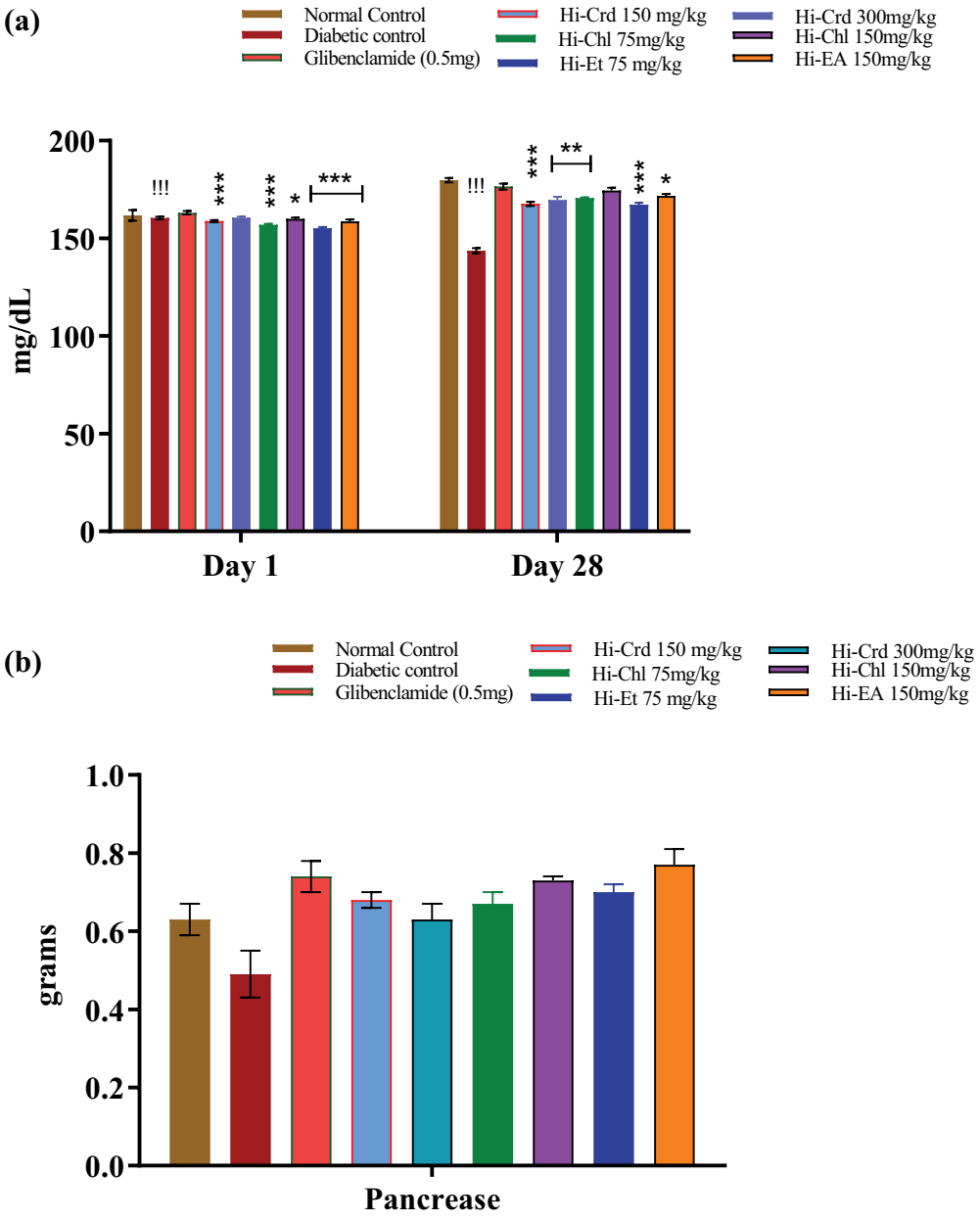

Figure 3: (a) Effect of Hi-Crd and fractions on the weight of animals. (b) Weight of pancreas. Data are expressed as mean ± SEM with $n = 8$. One-way ANOVA and Dunnett's *post-hoc* test were used to determine statistical significance. !!!$P < 0.001$ was considered significant compared to the control group. Within the diabetic group, differences were noted with significance levels of *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

5.27, 124.50 ± 2.59, and 106.0 ± 6.16 mg/dL. The level of HDL in animals of the diabetic control group decreased to 19.16 ± 3.37 compared to the normal control group (36.5 ± 1.99 mg/dL). Animals treated with Hi-Crd and fractions at respective doses produced a reversal response by increasing the HDL level ($P < 0.01$, $P < 0.001$, $n = 8$). At 150 and 300 mg/kg Hi-Crd, the level of HDL was 34.83 ± 2.49 and 35.33 ± 1.75 mg/dL. Animals treated with Hi-Chl and Hi-Et at respective doses produced similar results and increased HDL compared to the diabetic group, indicating beneficial approach of plant in hyperlipidemic response. The level of LDL in animals of the diabetic control group increased to a level of 44.50 ± 4.16 mg/dL compared to the normal control group of 17.66 ± 3.95 mg/dL, indicating a rise in the level of bad cholesterol. This negative effect was countered by treating the animals with Hi-Crd and its fractions. Animal treated with 75 and 150 mg/kg Hi-Chl and Hi-Et normalized the levels of LDL to 291.12 ± 1.08, 27.61 ± 1.92, 34.81 ± 3.94, and 31.32 ± 3.17, respectively.

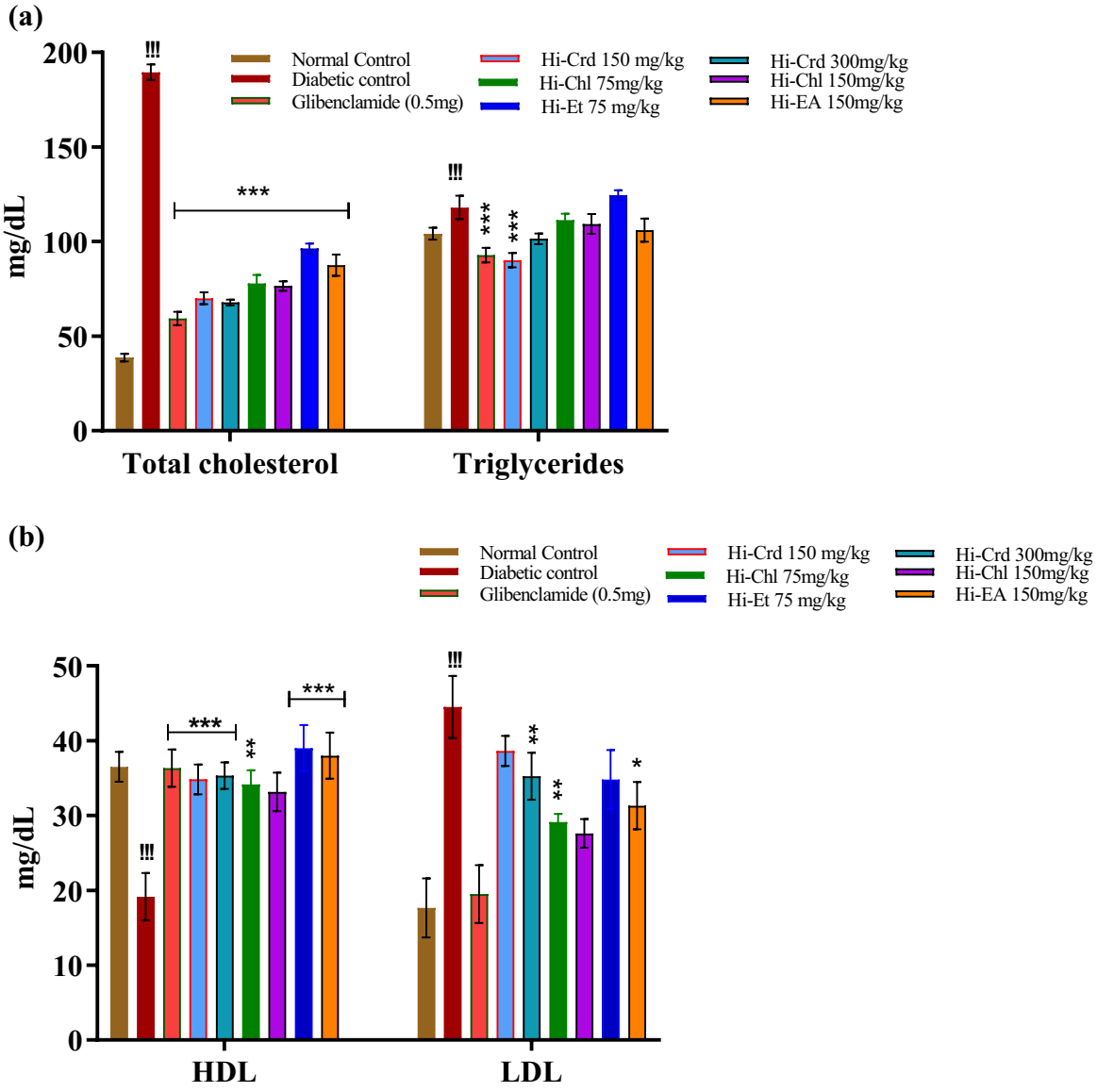

Figure 4: (a) Total cholesterol and triglycerides levels of diabetic animals. (b) HDL and LDL levels of diabetic animals. Anti-hyperlipidemic activity of *H. isora* is shown. Data are expressed as mean ± SEM with $n = 8$. One-way ANOVA and Dunnett's *post-hoc* test were used to determine statistical significance. !!!$P < 0.001$ was considered significant compared to the control group. Within the diabetic group, differences were noted with significance levels of *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

3.8 Histopathological examination of vital organs

Vital organs such as the kidney and liver were subjected to histopathological studies, and photomicrographs were performed using a microscope.

3.9 Photomicrographs of the kidney section of rats

Tissues of the kidney: Bowman's space, proximal and distal convoluted tubules, glomerulus, and Bowman's capsule are affected by paracetamol poisoning. Glomerulus is damaged

by inflammation and oxidative stress that decreases filtration and causes renal failure, as Bowman's capsule is also affected and produces a defect in processing of filtrates.

Proximal and distal convoluted tubules are disrupted by paracetamol poisoning that alters reabsorption and imbalances of electrolytes. Renal filtration was impeded by injury to Bowman's space that disturbed the flow of urine. Paracetamol poisoning caused structural changes in kidneys and produced functional alterations. In the current study, when standard drugs and extracts of *H. isora* were administered to rats, it successfully lowered paracetamol toxicity in kidney tissue (Figure 5). Glomerulus, proximal, and distal convoluted tubules and Bowman's capsule regained their structure and function upon treatment with standard drug and plant extract (150 and 300 mg/kg b.w.). The treatment reduced oxidative stress and inflammation and promoted repair of the renal tissues. The volume and composition of urine were improved upon treatment, and the renal function and glomerular filtration were restored to normal.

Renal homeostasis and electrolyte balance were restored by plant extracts and improved the function of the proximal and distal convoluted tubules. Plant extract and standard drug effectively reversed the toxic effects of paracetamol on kidney tissues and promoted recovery of renal function to a healthy level. The kidney function tests

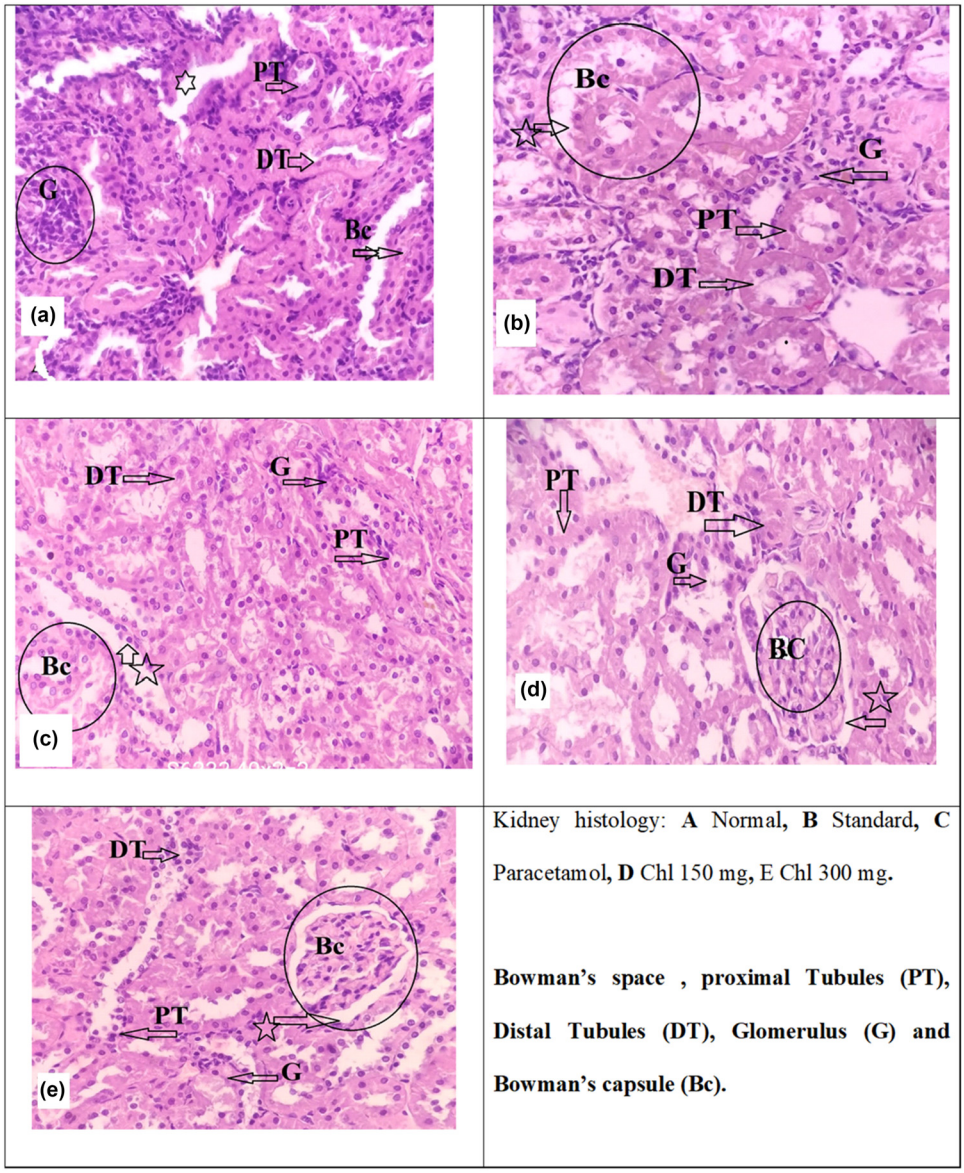

Figure 5: Protective effect of *H. isora* on paracetamol-induced toxicity in kidneys. (a) Normal; (b) Standard; (c) Paracetamol; (d) Chl 150 mg/kg body weight and (e) Chl 300 mg/kg body weight.

confirmed the healing effects of the plant extract on the kidney histology and functions.

3.10 Photomicrographs of the liver section of rats

Hepatocytes are the primary cells of the liver that may be affected by overdosing of paracetamol. The metabolism of drugs in hepatocytes is the main mechanism underlying paracetamol-induced hepatotoxicity. In the liver, cytochrome P450 enzymes, especially CYP2E1, forms *N*-acetyl-p-benzoquinone imine (NAPQI) during the metabolism of paracetamol. Under normal conditions, glutathione (an antioxidant in the liver) detoxifies NAPQI. Due to overdosing on paracetamol, more NAPQI is produced that glutathione cannot store, which damages hepatocytes. The slides show that inflammation was caused in hepatocytes by paracetamol, which produced oxidative damage and even dead tissues. In biliary system, bile ducts are vital parts and carry bile from liver to gut for digestion. The elimination of wastes may also be affected by paracetamol-induced hepatotoxicity.

Damages caused to the liver by paracetamol interfered with bile ducts and regular flow of bile. Injury to the epithelial cells of bile ducts is caused by inflammation, oxidative stress, and toxic metabolites and hinders both secretion and the flow of bile. In the sinusoids of liver, specialized macrophages called Kupffer cells (involved in the immunological defense mechanism of the liver) are present and are affected by paracetamol poisoning. Liver damages are caused by paracetamol due to the inflammatory reaction that releases chemokines and pro-inflammatory cytokines. Kupffer cells produced cytokines like interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) and are actively implicated in this inflammatory cascade. This inflammatory environment accelerated the development of hepatotoxicity and worsened liver damage. An increase in the cell count was noted during paracetamol dosing.

The hepatic artery and portal vein (vital parts of the liver's vascular system) were also affected by paracetamol poisoning, resulting in hepatic ischemia, thrombosis, vascular dysfunction, and portal hypertension. Endothelial damage, thrombus formation, and vasoconstriction experienced by these cells were due to oxidative stress and inflammation caused by a paracetamol overdose. Prolonged vascular compromise resulted in hepatic ischemia, which further worsened liver injury by depriving of nutrients and oxygen. Resistance to blood flow was noted that developed portal hypertension.

This inflammatory environment damaged the liver and increased the development of hepatotoxicity (Figure 6). However, these symptoms were improved by treating with plant extracts (*H. isora* at 150 and 300 mg/kg b.w. doses) and standard drug. Treatment successfully reduced inflammation, restored normal bile flow, and enhanced Kupffer cell activity and ultimately promoted recovery of the liver tissues. Signs of improvements, like enhanced blood flow, decreased thrombosis, and resolution of vascular dysfunction, were noticed in the hepatic artery and portal vein. These developments resulted in the recovery of the liver function and the alleviation of problems related to paracetamol toxicity. The liver function tests confirmed the progressive effects of the plant extract on the liver histology and functions.

4 Discussion

Medicinal plants from the very beginning of human life on Earth have been explored for various therapeutic actions. Being nature factories of primary and secondary metabolites, they have provided food, shelter, and medication not only to humans but also to other primates; they are still under constant exploration to find effective therapeutic agents for the emerging diseases [25]. The geographic location and weather of Pakistan is unique, which supports animals and plants mega biodiversity. Being a third world country, most of its resources are unexplored. Scientists at present are actively exploring its flora for medicinal potentials [26]. This has opened a new gateway for the researchers to evaluate basic components of herbal medication. Consequently, researchers have prioritized exploration of plant families and species that exhibit therapeutic potentials, especially anti-diabetic potency involving inhibition of α-glucosidase [27]. *Helicteres isora* is a plant native to various regions of Asia, including South China, Malay Peninsula, Java, Indian Subcontinent, Saudi Arabia, and Australian forests. It is known for its abundant content that is essential from a nutraceutical point of view such as proteins, carbohydrates, antioxidants, fiber, iron, calcium, and phosphorus. The active phytochemicals present in *H. isora* are caffeic acid, gallic acid, p-coumaric acid, and vanillin; in addition, isocucurbitacin b and cucurbitacin b are also present in the roots of *H. isora.* Moreover, Satake et al. (1999) identified several compounds isolated from *H. isora*, including isoscutellarein and its derivatives, rosmarinic acid and its derivatives, D-glucopyranosyl isorinic acid with rosmarinic acid, helisterculins A and B, and helisorin. These compounds contribute to the plant's potential health benefits and medicinal properties. Utilization of

glucose in L-6 cells was investigated by *in vitro* studies, and the results indicate that extracts derived in hot water from the fruit of *H. isora* produced a significant enhancement in the uptake of glucose by an increase of 28.99% from that of the control group at a dose of 200 µg/mL [28].

Since ancient times, humans have been utilizing extracts and secondary metabolites derived from plants for medicinal purposes, and more often, secondary metabolites are particularly valued and are often more effective and well-tolerated within the living system compared to

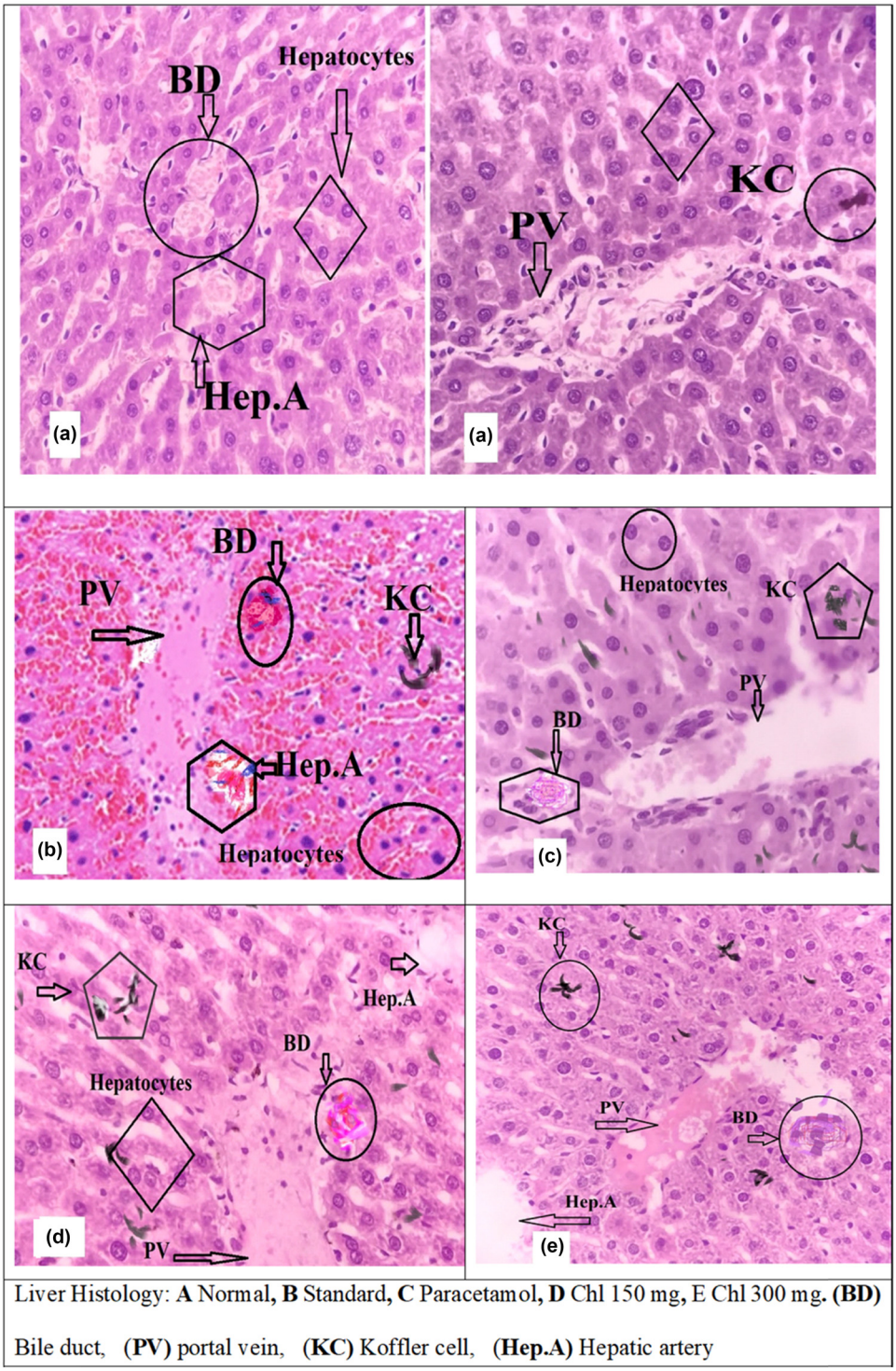

Figure 6: Protective effect of *H. isora* on paracetamol-induced toxicity in the liver. (a) Normal; (b) Standard; (c) Paracetamol; (d) Chl 150 mg/kg body weight and (e) Chl 300 mg/kg body weight.

synthetic compounds [29]. The use of medicinal plants is commanding due to their affordability and lower incidence of side effects, making them a valuable and accessible option for healthcare in these communities.

Research in phytochemistry has found the presence of valuable compounds in plants, like alkaloids, triterpenes, polyphenols, sterols, and flavonoids. These phytoconstituents showed valued pharmacological properties such as antidiabetic, antioxidant, and anticholinesterase effects. Their potential in providing beneficial health effects has been extensively studied and recognized.

Extracts from *H. isora* crude (Hi-Crd) and its fractions (Hi-Chl and Hi-Et) were screened *in vitro* for the inhibition of alpha-glucosidase. Among these, Hi-Chl fraction significantly inhibited alpha-glucosidase with an IC_{50} value of 122.63 µg/mL. The Hi-Crd and fractions were evaluated for their *in vivo* potential in the management of diabetes. Hi-Crd at 150 and 300 mg/kg lowered blood glucose levels from 503.10 ± 3.80 to 151.6 ± 3.91 and 119.1 ± 2.01 mg/dL, respectively. Among the fractions, Hi-Chl at 150 mg/kg lowered the blood glucose level up to 125.6 ± 3.72 mg/dL.

In the diabetic model, alteration in biomarkers of hyperlipidemia like cholesterol, triglycerides, LDL, and HDL levels was documented compared to the normal group. Treating animals with Hi-Crd, fractions, and standard glibenclamide reverted the changes in the level of biomarkers compared to the diabetic control group. Anti-hyperglycemic activity performed on the roots of extracts from *H. isora* was explored and the results showed that extracts (aqueous and butanol) at doses of 250 mg/kg for about 10 days in alloxan-induced diabetic rats experienced a decrease in blood glucose levels by 51.14, and 69.13%, respectively [22].

The liver and kidney profiling showed that the chloroform fraction Hi-Chl at 75 and 150 mg/kg b.w. doses prominently normalized all the relevant tested parameters in comparison to the negative control group. The level of creatinine was decreased by alloxan used to induce diabetes in the animal model, which was normalized by the selected plant extracts. In the same way, the blood urea level was regularized throughout the study period by the extracts. The most potential activity was noted for Hi-Chl at both tested doses. A similar study conducted by Kumar et al. and Shah et al. affirms that the plant extract has potential hepato-protective and nephron-protective activity [16,30]. It was concluded in the *in vitro* as well as *in vivo* study that *H. isora* extract and fractions had therapeutic potential and could be used for anti-diabetic purposes. The extracts reversed weight loss and reduced blood glucose levels effectively. The extracts delivered positive effects on the liver profile, and lowered the levels of cholesterol and triglycerides. The study suggests that the extracts of *H. isora*, specifically its Hi-Chl fraction, have considerable antidiabetic potential and can, therefore, be considered to contain anti-diabetic constituents, which need to be explored further. The plant has also the potential to treat toxicity.

5 Conclusions

This study analyzed the phytochemical composition of the chloroform fraction of *H. sora* using GC-MS analysis. It also focused on evaluating the hepatic and nephron-protective effects of the selected plant as well as the anti-diabetic potential in animal models. A number of secondary metabolites were identified through the chromatographic technique used. The fluctuations in liver and kidney biomarkers caused by the utilized toxin (paracetamol) were normalized by the extract, indicating the hepatic and nephroprotection effects of the extracts. Substantial antidiabetic activity was also observed. This study is limited to only animal models and no clinical documentations have been made in humans. The studied hepato and nephro toxicity and antidiabetic have been documented for only animal models, and further studies are encouraged to extend its use to human after validation of the present findings in other animal models.

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Author contributions: HB and MAK: conceptualization, validation, software, and writing the original draft; SWAS and MZ: project administration, conceptualization, and visualization; AK and MG: visualization, investigation, and software, validation, writing, review and editing; RU and ZI: formal analysis and investigation; MZ: data validation, visualization, and editing; ABS and AK: funding acquisition and technical assistance. All authors read and approved the final manuscript.

Conflict of interest: The authors have no conflict of interest.

Ethical approval: This research study was carried out as per Departmental Ethical Committee University of Malakand in compliance with the 2008 Animal Bye-Laws.

Data availability statement: All the data are presented in this manuscript. There are no associated data in any repository.

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