

Antioxidant and Hepatoprotective Potential of Swaras and Hima Extracts of *Tinospora cordifolia* and *Boerhavia diffusa* in Swiss albino Mice

Amit Kaushik^{1,2,#}, Athar Husain^{1,2,#}, Harshika Awasthi¹, Dewasya Pratap Singh¹, Raziuddin Khan², Dayanandan Mani¹

¹Herbal Medicinal Products Department, CSIR-Central Institute of Medicinal and Aromatic Plants, ²Mahatma Gandhi Institute of Pharmacy, Lucknow, Uttar Pradesh, India

[#]These authors have equally contributed to this work.

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ABSTRACT

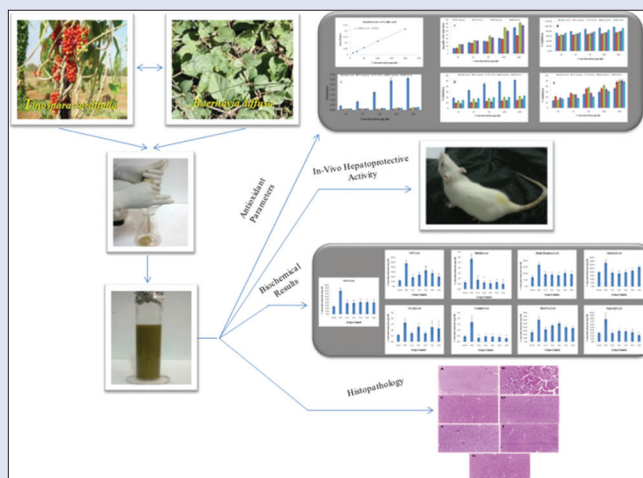
Background: In *Ayurveda*, five basic extraction procedures are mentioned in order of their decreasing potency. Swaras is considered as the most potent followed by, *kalka*, *kwatha*, *fanta* and *hima*. **Objective:** Present study was carried out to investigate the antioxidant and hepatoprotective potential of swaras and hima extracts of *T. cordifolia* and *B. diffusa*. **Materials and Methods:** Swaras and hima extracts of *T. cordifolia* and *B. diffusa* were prepared. Phytochemical screening and *in vitro* antioxidant activities was carried out using standard methods. Hepatoprotective efficacy of extracts were carried out in *Swiss albino* mice using paracetamol induced hepatotoxicity. Animals were administered with swaras and hima extracts of both plants at 200 mg/kg BW dose for 7 days and on 8th day hepatotoxicity was induced by intraperitoneal injection of paracetamol at 500 mg/kg BW. The degree of liver protection was determined by measuring the levels of liver enzymes followed by histopathology. **Results and Discussion:** The results of phytochemical, antioxidant and hepatoprotective activities showed that there were no significant difference between swaras and hima extracts. Both the extract of *T. cordifolia* were equally potent in reducing SGOT ($P < 0.01$) and ALP level ($P < 0.001$). Similar effects were observed with the Swaras and hima extracts of *B. diffusa*. Both the extracts reduced SGOT and ALP ($P < 0.01$). Histopathological findings among all the extracts were also more or less similar in lowering the paracetamol mitigated necrosis.

Conclusion: The present study suggested that *T. cordifolia* and *B. diffusa* possess potential hepatoprotective activity irrespective of the extraction procedure.

Key words: Antioxidant, *Ayurveda*, hepatoprotective, hima, histopathology, swaras

SUMMARY

- Aqueous extracts of *Tinospora cordifolia* and *Boerhavia diffusa* exhibited significant antioxidant and hepatoprotective activities
- Aqueous extracts of both the plants were extracted using different extraction procedures mentioned in *Ayurveda*
- Swaras and hima extracts of both the plants significantly reduced the deleterious effects of paracetamol, suggesting that both the plant extracts are equipotent
- Acute toxicity of both the plant extracts did not produce any toxic effects.



Abbreviations used: TC swaras: *T. cordifolia* swaras; TC hima: *T. cordifolia* hima; BD swaras: *B. diffusa* swaras; BD hima: *B. diffusa* hima; BW: Body weight; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; SGOT: Serum glutamate oxaloacetate transaminase; SGPT: Serum glutamate pyruvate transaminase; ALP: Alkaline phosphatase; I.P: Intraperitoneal; TAC: Total antioxidant capacity; DPPH: 2,2-diphenyl-1-picrylhydrazyl; TCA: Trichloro acetic acid; NO: Nitric oxide; TPC: Total phenolic content; NAPQI: N-acetyl-p-benzoquinone imine; PCM: Paracetamol.

Correspondence:

Dr. Dayanandan Mani,
Herbal Medicinal Products Department,
CSIR-Central Institute of Medicinal and
Aromatic Plants, Kukrail Picnic Spot Road,
Lucknow - 226 015, Uttar Pradesh, India.
E-mail: drdnmani@gmail.com;
dn.mani@cimap.res.in
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INTRODUCTION

Liver plays a vital role in various functions of the body such as metabolism, secretion, storage, and detoxification of endogenous and exogenous substances. Liver diseases are the major health problem worldwide due to unhealthy diet, pollution, environmental toxicants, infections, and sedentary lifestyle.^[1] Paracetamol, a well known analgesic and antipyretic drug metabolizes in liver and produces harmful metabolites and free radicals that cause oxidative stress.

Medicinal plant-based drugs are considered as a potent therapeutic agent to reduce oxidative stress and help in the treatment of liver diseases. They contain various phytomolecules such as flavonoids and phenolic

compounds, which are helpful in free radical scavenging, reduce oxidative stress, and help in regeneration of hepatocytes. Extractions

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of phytochemicals/bioactive agents from medicinal plants are well described in *Ayurveda* based on the potency known as “*Panch vidhi kasaya kalpana*.” “*Swarasa*,” the expressed juice of fresh plant; “*Kalka*,” fine paste of fresh plant; “*Kwaatha*,” the decoction; “*Faanta*,” hot water infusion; and “*Hima*” or “*Sheeta*,” the cold water infusion. According to *Ayurveda*, *swarasa* is the most potent extract of any plant while *hima* is least potent.^[2] *Tinospora cordifolia* (TC), commonly known as “*Guduchi*,” belongs to family Menispermaceae. Aqueous extract of *T. cordifolia* is widely used as household medicine in liver diseases. *Boerhavia diffusa*, belongs to family Nyctaginaceae, is commonly known as “*Punarnava*.” In *Ayurveda* and *Unani* systems, *B. diffusa* has been reported to be used in the treatment of jaundice and hepatitis.^[3,4]

In the present study, *swaras* extract of both these plants was compared to the *hima* extracts. To the best of our knowledge, no previous study was conducted to validate the *panch vidhi kasaya kalpana*. The aim of the present study was to check the potency of both the plant extracts to substantiate the classical *panch vidhi kasaya kalpana*.

MATERIALS AND METHODS

Plant materials

The fresh stems of *T. cordifolia* and whole plant of *B. diffusa* were collected and authenticated by the Department of Botany and Pharmacognosy, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow.

Preparation of extracts

T. cordifolia and *B. diffusa* (italics) were freshly collected from farm of CSIR-CIMAP and *Swaras* was made after washing and grinding the plant materials. *Hima* extract was prepared by soaking the shade-dried plant material overnight in distilled water and filtered using muslin cloth.^[5] Thereafter, extracts were concentrated on a rotary evaporator (BUTCHI; R-210, Switzerland) and lyophilized (Labconco, Fort Scott, KS, USA). The extract was then standardized as per the WHO guidelines.^[6]

Preliminary phytochemical screening

The preliminary phytochemical study of the lyophilized extract was done by standard qualitative methods.^[7]

Antioxidant activity

Total antioxidant capacity

One hundred microliter of different concentrations of samples (10–200 µg/ml) reacted with 1 ml TAC reagent (0.3 N sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate), incubated at 95°C for 90 min, and the absorbance was taken at 695 nm against blank.^[8]

2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity

One hundred microliter of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (0.1 M in methanol) was added to 400 µl test samples. The mixture was shaken and incubated under dark for 30 min at room temperature. Absorbance was taken at 517 nm.^[9]

Reducing power estimation

Samples of 200 µl were mixed with 200 µl phosphate buffer (300 mM, 6.6 pH) and 200 µl potassium ferricyanide (1% w/v). The mixture was incubated at 50°C for 20 min, cooled, and 200 µl of trichloro acetic acid (TCA, 10% w/v) was added. Then, the mixture was centrifuged at 3000 rpm for 5 min and 100 µl of the upper layer was collected. One hundred µl of double-distilled water and 20 µl of FeCl₃ (0.1% w/v) were added, and absorbance was taken at 700 nm against blank.^[10]

Nitric oxide radical scavenging activity

Two hundred microliter of 10 mM sodium nitroprusside was dissolved in 0.5 ml phosphate buffer saline (pH 7.4), mixed with 25 µl of sample, and incubated for 150 min. Fifty microliter of the incubated mixture was mixed in 100 µl sulfanilamide (1% in 5% phosphoric acid), and incubated for 5 min. Exactly 100 µl of 0.1% (α-naphthyl)-ethylene diamine was added to the reaction mixture, incubated for 30 min, and the absorbance was measured at 546 nm.^[11]

Hydroxyl radical scavenging activity

Fifty microliter sample was mixed with 50 µl of FeSO₄·7H₂O (10 mM), EDTA (10 mM), 2-deoxyribose (10 mM), and 250 µl phosphate buffer (0.1 M, 7.4 pH). Exactly 50 µl of H₂O₂ (10 mM) was added to the reaction mixture and incubated at 37°C for 4 h. Exactly 250 µl each of TCA (2.8%) and thiobarbituric acid (1%) was added and boiled for 10 min, cooled, and the absorbance was measured at 520 nm.^[12]

Total phenolic content

Ten microliter samples were mixed with 100 µl FCR (10% v/v) and 80 µl sodium carbonate (7.5%). The mixture was incubated at 40°C for 30 min.^[13] The absorbance was measured at 765 nm using the equation obtained from a standard gallic acid calibration curve [Figure 1].

Acute toxicity study

Swiss albino mice (20–25g) were divided into three groups of six animals in each group. All the test extracts were administered orally at single doses of 2000 mg/kg body weight (bw), following the guidelines of OECD-423.^[14]

In vivo hepatoprotective activity

Test animals

Forty-two female *S. albino* mice weighing 25–30 g were obtained from CSIR-CIMAP, Lucknow. Animals were acclimatized at standard laboratory condition, fed with standard pellet diet (Dayal Industries, Lucknow, India) and water *ad libitum*. Institutional Animal Ethics Committee constituted under the CPCSEA, Government of India, New Delhi, approved the protocol of the experiment (AH-2012-10).

All the extracts were dissolved in distilled water and administered orally through intragastric tube daily for 7 days. Paracetamol overdose was used to induce liver toxicity in mice. Animals were randomly divided into seven groups of six animals each;

- Group 1: Vehicle control, received distilled water orally
- Group 2: Negative control, received distilled water
- Group 3: Positive control, pretreated with Liv-52 for 7 days at 5.2 ml/kg.bw

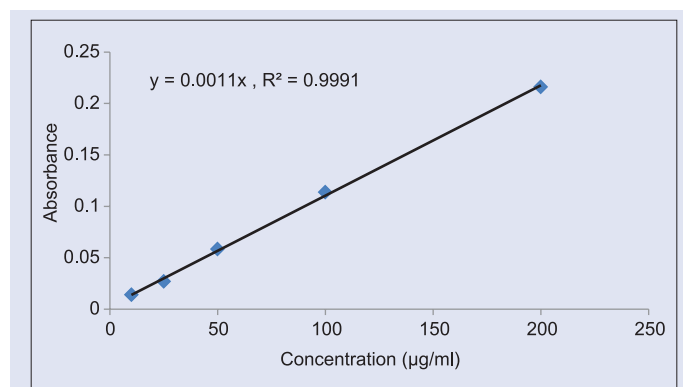


Figure 1: Standard curve of gallic acid

- Groups 4, 5, 6, and 7 served as test groups. Group 4 was pretreated with *T. cordifolia swaras* and Group 5 with *T. cordifolia hima* at 200 mg/kg.bw
- Similarly, Group 6 was pretreated with *B. diffusa swaras* and Group 7 with *B. diffusa hima* at the dose of 200 mg/kg.bw.

On the 8th day, all the groups except Group 1 were given an intraperitoneal (IP) injection of paracetamol at 500 mg/kg.bw. After 2:30 h of paracetamol administration, 500 μ l blood was collected from retro-orbital plexus. The serum was separated and stored at -20°C for experimental analysis.

Histopathological studies

Animals were sacrificed; liver was dissected out and washed with phosphate buffer (pH 7.4) for complete removal of blood stains and clot. Tissue was fixed in 10% formalin and embedded in molten paraffin wax followed by cutting of sections with microtome. Deparaffinized sections were then stained with hematoxylin and eosin and observed under a microscope for histopathological changes.^[15]

Biochemical analysis

Serum was analyzed for different biochemical parameters using standard diagnostic kits as per manufacturer's protocol (Labkit from Merck Chemicals Private Ltd.).^[16]

Statistical analysis

Statistical analyses were performed with the help of Instat GraphPad software (version 3.0, La Jolla, CA 92037 USA). Difference between means was calculated by one-way analysis of variance followed by Student–Newman–Keuls test as *post hoc* test ($n = 6$ mice/group). Results were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Swaras and *hima* extracts of *T. cordifolia* and *B. diffusa* showed the presence of major chemical compounds [Table 1].

Antioxidant activity

The total antioxidant capacity of test extracts was increased with concentration [Figure 2a]. Free radical scavenging activity of all the test extracts was expressed in terms of percentage inhibition of DPPH radical. At all the concentrations of test solution, free radical was more or less inhibited [Figure 2b]. The IC_{50} values of *swaras* and *hima* extracts of *T. cordifolia* and *B. diffusa* are 57.77, 55.36, 53.43, and 48.38 $\mu\text{g}/\text{ml}$, respectively, as compared to gallic acid (44.96 $\mu\text{g}/\text{ml}$). Both the plant extracts did not exhibit any reducing power activity in comparison to gallic acid [Figure 2c]. Similarly, none of the test extracts scavenge nitric oxide radical (*T. cordifolia*: 309.77 and 214.84 $\mu\text{g}/\text{ml}$ and *B. diffusa*: 310.27

and 204.54 $\mu\text{g}/\text{ml}$) in comparison to standard gallic acid (97.82 $\mu\text{g}/\text{ml}$) [Figure 2d]. However, all the test extracts showed scavenging activity against hydroxyl radical in a concentration-dependent manner. IC_{50} values of *T. cordifolia* extracts were 114.82 and 115.22 $\mu\text{g}/\text{ml}$ and *B. diffusa* extracts were 123.53 and 145.13 $\mu\text{g}/\text{ml}$, as compared to gallic acid, 156.58 $\mu\text{g}/\text{ml}$ [Figure 2e]. Total phenolic content was found to be 25.7, 29.70, 30.75, and 28.90 mg of gallic acid equivalent per gram dry weight for *T. cordifolia* and *B. diffusa*, respectively. These results showed that *swaras* and *hima* extracts of *T. cordifolia* and *B. diffusa* are equally potent in the inhibition of free radical generation.

Acute toxicity study

Oral administration of *swaras* and *hima* extracts of both the plants did not produce mortality in experimental mice at 2000 mg/kg.bw. Oral administration of all the extracts did not show noticeable change in body weight, food intake, and general behavior compared to the control group [Table 2].

Hepatoprotective activity

The hepatoprotective efficacy of the *swaras* and *hima* extracts against paracetamol-induced hepatotoxicity is shown in Table 3. Paracetamol at 500 mg/kg IP significantly ($P < 0.05$) elevated the serum levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase, bilirubin, total cholesterol, triglyceride, low-density lipoprotein (LDL), creatinine, urea, and uric acid, which confirmed that the paracetamol causes liver injury as well as disturbs the lipid profile and kidney functions at higher doses. On the other hand, the HDL level was decreased significantly. Pretreatment with Liv-52 syrup (5.2 ml/kg.bw) and *swaras* and *hima* extracts (200 mg/kg.bw) significantly ($P < 0.001$) decreases the elevated enzyme levels to normal level. The restoration effect of *T. cordifolia* and *B. diffusa* extracts is comparable with that of the standard drug Liv-52 syrup.

Biochemical marker levels were measured to evaluate liver injury.^[17] Paracetamol-induced hepatotoxicity damages plasma membrane of hepatocytes which disturbs the transport mechanism of hepatocytes, leading to an increase in liver enzymes in blood circulation.^[18] Treatment with Liv-52 syrup and *swaras* and *hima* extracts restored the increased levels of SGOT and SGPT likely by the repair of hepatic cell damage and stabilization of plasma membrane.^[19] Increased ALP level is an indication of cellular cholestasis.^[20] ALP level was significantly reduced in extract treated groups which indicate that plant extracts significantly protected the liver from paracetamol induced toxicity. Hyperbilirubinemia may occurs due to the over production of bilirubin which damage and reduce the ability to excrete normal amounts of bilirubin from liver.^[21] The significant decrease in serum bilirubin level suggested the hepatoprotective potential of all the test extracts.

Paracetamol induced toxicity showed a significant ($P < 0.001$) increase in the levels of serum cholesterol, triglyceride, LDL, creatinine, urea, uric acid and decrease the level of HDL.^[22] Oxidative stress involves in generation of oxygen derived species or *N*-acetyl-*p*-benzoquinone imine free radical, initiates lipid peroxidation.^[23] It was reported earlier that the hypolipidemic drugs with antioxidant properties may prevent LDL peroxidation.^[24] Increased serum LDL level was significantly reduced in *swaras* and *hima* extracts possibly due to their antioxidant property.^[25] Paracetamol induced kidney damage was expressed by reduction in glomerular filtration rate which results increased levels of creatinine, urea and uric acid in serum. Oral administration of both *swaras* and *hima* extract significantly ($P < 0.001$) attenuated serum cholesterol, triglyceride, LDL, creatinine, urea and uric acid concentrations and increased the HDL concentrations compared to toxic control group. At 200 mg/kg, *swaras* and *hima* extract increased the HDL concentration, lowered the cholesterol and other parameters concentrations more than

Table 1: Preliminary phytochemical screening

Phytochemical tests	TC <i>swaras</i>	TC <i>hima</i>	BD <i>swaras</i>	BD <i>hima</i>
Alkaloids	+	+	+	+
Cardiac glycosides	+	+	+	+
Flavonoids	+	+	+	+
Saponin	+	+	+	+
Steroids	+	+	+	+
Tannins	+	+	+	+
Phenolics	+	+	+	+
Triterpenoids	+	+	+	+

+: Present

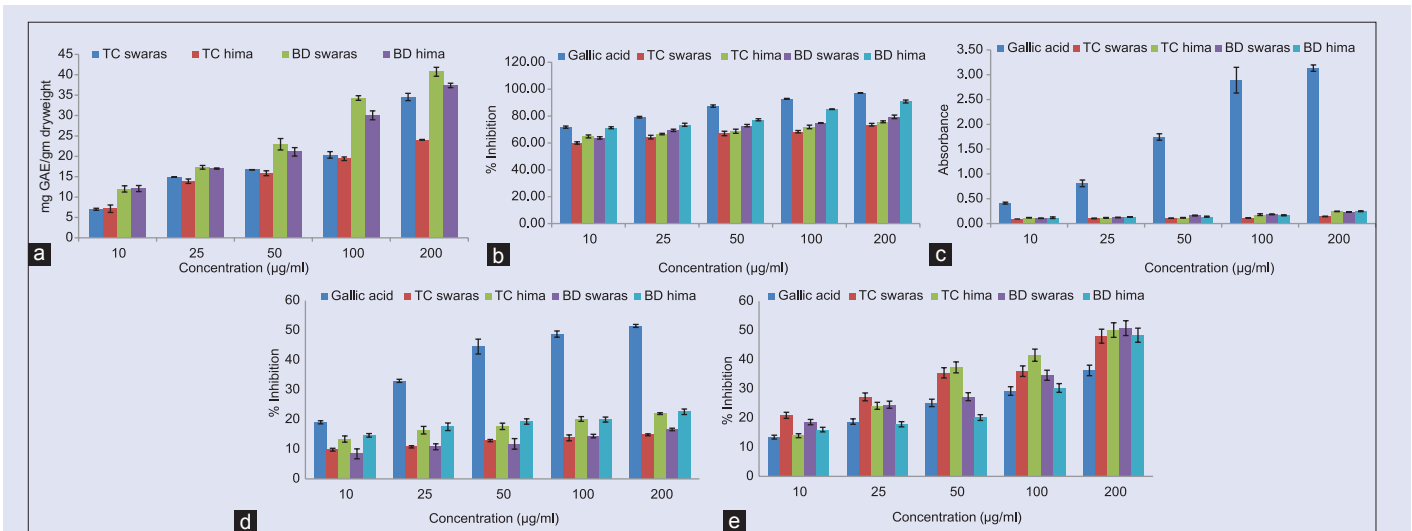


Figure 2: *In vitro* antioxidant activity: (a) total antioxidant capacity, (b) against 2,2-diphenyl-1-picrylhydrazyl radical, (c) reducing power estimation, (d) against nitric oxide radical, (e) against H₂O₂ radical

Table 2: Acute toxicity study on biochemical parameters

Parameters/groups	Control	TC swaras (2000 mg/kg)	TC hima (2000 mg/kg)	BD swaras (2000 mg/kg)	BD hima (2000 mg/kg)
Body weight (g)	26.5±0.78	22.81±0.68	24.78±0.66	24.20±1.20	22.15±1.30
Haemoglobin (g/dL)	12.35±0.82	12.68±0.52	12.20±0.80	12.20±0.68	11.20±0.40
SGOT (U/L)	26.11±4.53	29.32±6.22	26.25±4.18	25.68±1.15	23.78±1.05
SGPT (U/L)	24.44±3.45	20.52±2.70	28.50±4.55	22.30±6.18	21.60±7.32
ALP (U/L)	231.46±14.95	230.12±13.25	240.24±9.50	220.40±30.98	205.20±40.96
Bilirubin (mg/dl)	0.63±0.16	0.50±0.12	0.40±0.16	0.60±0.20	0.49±0.29
Cholesterol (mg/dl)	78.98±3.78	57.82±2.56	55.20±4.58	75.40±4.12	80.20±2.25
Triglycerides (mg/dl)	130.13±2.42	125.22±20.14	135.25±30.16	110.44±9.62	98.68±13.80
HDL (mg/dl)	77.75±5.78	89.94±6.44	70.66±4.40	86.59±4.15	86.60±4.10
LDL (mg/dl)	79.74±6.79	90.92±3.40	92.88±2.68	85.48±5.25	85.16±5.30
Creatinine (mg/dl)	0.46±0.06	0.43±0.32	0.40±0.37	0.49±0.34	0.38±0.47
Blood urea (mg/ml)	25.91±6.91	26.94±0.12	22.58±0.14	31.86±0.16	32.12±0.5

n=6; values are represented as mean±SEM. ALP: Alkaline phosphatase; SGOT: Serum glutamate oxaloacetate transaminase; SGPT: Serum glutamate pyruvate transaminase; HDL: High density lipoprotein; LDL: Low density lipoprotein; SEM: Standard error of mean; TC: *Tinospora cordifolia*; BD: *Boerhavia diffusa*

Table 3: Effect of swaras and hima extracts on serum biochemical parameters

Biochemical marker enzymes	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
	Vehicle control	Paracetamol	Positive control	TC swaras (200 mg/kg)	TC hima (200 mg/kg)	BD swaras (200 mg/kg)	BD hima (200 mg/kg)
SGOT (IU/L)	26.11±4.53	80.51±7.49***	37.98±6.08***	38.73±6.52***	41.34±4.70***	40.76±4.46***	39.81±4.22***
SGPT (IU/L)	24.44±3.45	95.87±6.73***	37.15±4.22***	48.15±8.88**	67.00±10.49ns	54.98±8.84**	40.28±12.51**
ALP (IU/L)	71.46±14.95	166.59±11.98***	94.73±12.24***	90.75±10.65***	85.54±8.63***	102.38±9.30**	95.72±1.91***
Bilirubin (mg/dl)	0.63±0.16	2.84±0.36***	0.88±0.25***	0.62±0.18***	0.53±0.10***	0.65±0.10***	0.49±0.24***
Cholesterol	78.98±3.78	130.46±9.89***	75.59±6.08**	71.34±10.98***	78.63±5.93***	87.12±5.18**	107.87±8.55**
Triglyceride	30.13±2.42	78.16±15.40***	29.94±4.28**	37.03±4.03**	35.64±2.24**	32.51±6.47***	22.74±3.86***
Creatinine	0.46±0.06	1.69±0.40***	0.33±0.03***	0.46±0.04***	0.42±0.03***	0.39±0.07***	0.29±0.04***
Blood urea	25.91±6.91	60.51±8.52***	33.88±7.23***	44.62±4.06**	50.83±5.20*	39.95±4.34**	37.43±6.07*
Uric acid	0.58±0.06	1.65±0.04***	0.72±0.15***	1.28±0.35*	0.72±0.15***	1.26±0.58*	1.13±0.68**

All the values are expressed as mean±SEM (n=6), Group 2 (negative control) was compared with Group 1 (vehicle control), Groups 3, 4, 5, 6, 7 (positive and test groups) was compared with Group 2 (negative control), level of significance at *P≤0.05; **P≤0.01; ***P≤0.001. SGOT: Serum glutamate oxaloacetate transaminase; SGPT: Serum glutamate pyruvate transaminase; ALP: Alkaline phosphatase; TC: *Tinospora cordifolia*; BD: *Boerhavia diffusa*; SEM: Standard error of mean

Liv-52. The rise in serum HDL concentrations and decrease in other lipid and kidney parameters suggests the stabilization and restoration of endoplasmic reticulum and kidney cells.

The hepatoprotective efficacy of the test extracts against paracetamol intoxication was further confirmed by histopathological examination of liver tissues [Figure 3].

CONCLUSION

In the present study, swaras and hima extracts of *T. cordifolia* and *B. diffusa* express similar effects which are in contrast to the literature probably due to the presence of similar nature of compounds. Therefore the study suggests that both the hima and swaras extracts of any plant show similar effects.

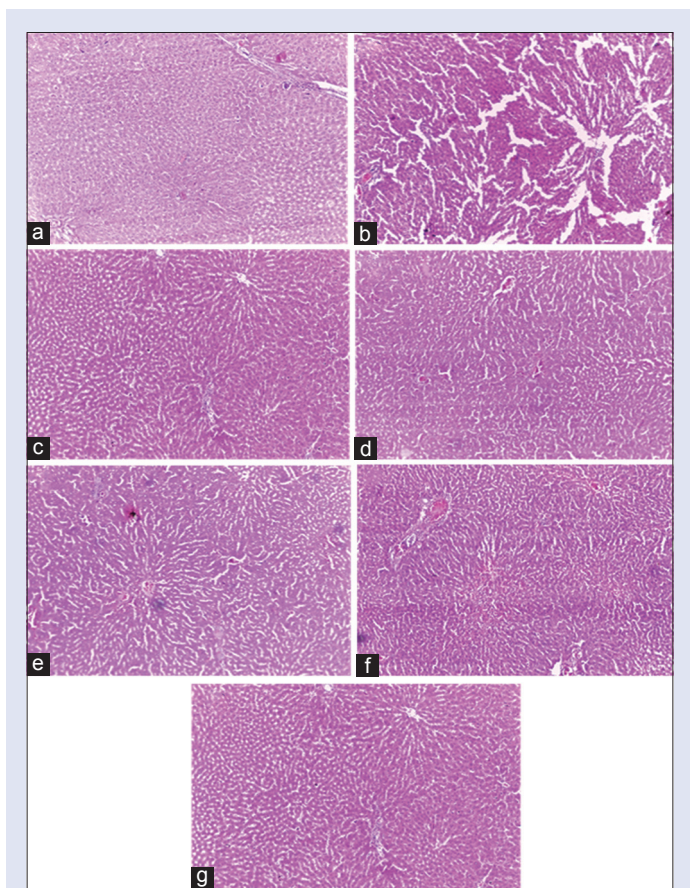


Figure 3: Histological observations (x40), (a) vehicle control (b) paracetamol (c) Liv. 52 (d) *Tinospora cordifolia* swaras (e) *Tinospora cordifolia* hima (f) *Boerhavia diffusa* swaras (g) *Boerhavia diffusa* hima

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Conflicts of interest

There are no conflicts of interest.

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