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A novel polyherbal formulation containing thymoquinone attenuates carbon tetrachloride-induced hepatorenal injury in a rat model

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ABSTRACT

Objective: To evaluate a novel polyherbal formulation (BSVT) containing the standardized extracts from the leaves of *Boerhavia diffusa*, *Solidago virgaurea*, *Vitex negundo*, and thymoquinone in CCl_4 induced hepatorenal toxicity in rats.

Methods: A total of 36 rats were divided into six groups including normal control, CCl_4 (2 mL/kg, *i.p.*), CCl_4 (2 mL/kg, *i.p.*) + Cystone® (750 mg/kg *p.o.*), CCl_4 (2 mL/kg, *i.p.*) + BSVT (25 mg/kg, *p.o.*), CCl_4 (2 mL/kg, *i.p.*) + BSVT (50 mg/kg, *p.o.*), and CCl_4 (2 mL/kg, *i.p.*) + BSVT (100 mg/kg, *p.o.*). All treatments were given for four weeks. Serum levels of aspartate transaminase, alanine transaminase, alkaline phosphatase, cholesterol, total protein, serum urea, blood urea nitrogen and creatinine were assessed. Superoxide dismutase, malondialdehyde, and glutathione peroxidase were evaluated in tissue homogenate. The histopathological study of liver and kidney tissues was also done.

Results: Aspartate transaminase, alanine transaminase, alkaline phosphatase, cholesterol, serum urea, blood urea nitrogen and creatinine were significantly elevated (P<0.001) while total protein was considerably reduced in the CCl_4 group as compared to the normal control (P<0.001), which indicated hepatorenal toxicity. In addition, superoxide dismutase and glutathione peroxidase activities were significantly decreased (P<0.001) while malondialdehyde levels were increased markedly (P<0.001). Treatment with BSVT formulation recovered these parameters towards a normal level in a dose-dependent manner.

Conclusions: BSVT formulation ameliorates the hepatorenal toxicity in a dose-dependent manner. Furthermore, clinical studies are required to confirm its efficacy.

KEYWORDS: *Boerhavia diffusa*; *Solidago virgaurea*; *Vitex negundo*; Thymoquinone; Cystone®; Carbon tetrachloride; Hepatorenal

1. Introduction

Biological magnification is one of the prime reasons for deteriorating the health of the entire biosystem, consequently,

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severe health issues can be envisaged globally in humans or animals. The illness caused is not limited to liver and kidney dysfunctions. Hepatorenal toxicity can also be induced by a number of pharmacological agents generally intended to treat the patients. Renal injury induced by drugs is a frequent adverse effect that is responsible for peaking morbidity and has raised the bars of healthcare utilization[1]. Drug-induced renal toxicity is identified as the main contributor to kidney disease, including but not limited to acute kidney injury and chronic kidney disease[2]. Moreover, prolonged use of drugs such as rifampicin and isoniazid (anti-tubercular), and others like analgesics and antipyretics has been found to cause hepatotoxicity[3,4]. Also, frequent self-medication can lead to such unwanted condition, and the matter of concern is that these disorders are hardly diagnosed in the initial stage, and hence lead to exacerbated conditions of hepatorenal damage.

Carbon tetrachloride (CCl_4), generally employed for dry-cleaning of fabrics and clothes, is an established agent that can cause hepatorenal toxicity by causing acute tubular necrosis and liver cirrhosis like condition[5]. CCl_4, a strong lipophilic nephrotoxin, is usually bound to lipid and protein, and its noxiousness depends upon the arrangement of trichloromethyl radical ($CCl_3^{\bullet}$), which binds with oxygen to form the more lethal trichloromethyl peroxyl radical ($CCl_3O_2^{\bullet}$). As per the reports of several studies, CCl_4 can cause the generation of reactive oxygen species (ROS) in numerous tissues other than the liver including the kidney, heart, lung, testis, cerebrum, and blood[6]. Moreover, CCl_4 influences the function of renal mitochondria, including the flux of calcium over mitochondrial membranes[7].

There is an expanding enthusiasm for natural antioxidants, *e.g.* polyphenols, present in therapeutic and dietary plants, which may help in alleviating oxidative stress. Endogenous antioxidants in therapeutic herbs may play a critical part as a defense against oxidative damage and ensure the biological functions of cells[8].

Thymoquinone (TQ), a flavonoid derived from seeds of *Nigella sativa* which is generally known as black cumin, is diversely used in traditional medicinal systems to treat various ailments[9,10]. It has a protective action on different kinds of tissues like heart, kidney[11], skin and liver[12,13]. In recent findings, it has been concluded that TQ has an ameliorative effect against oxidative stress and inflammatory responses[14,15].

The therapeutic potential of plants has been examined in the ongoing scientific advancements all through the world because they have potent antioxidant activities without severe adverse reactions and side effects. Our study is based on a novel polyherbal formula using the drug extract of *Boerhavia diffusa* (*B. diffusa*), *Solidago virgaurea* (*S. virgaurea*), *Vitex negundo* (*V. negundo*) and TQ. Sasikumar *et al*. reported in his study that *B. diffusa* exhibits nephroprotective effects in cisplatin-induced nephrotoxicity in rats[16]. *S. virgaurea* was reported to cure acute renal injury[17] and *V. negundo* was found to possess activity against chemically induced renal toxicity[18]. The major phytoconstituents of *B. diffusa* are punarnavine, carotenoids, and myricetin. *Nigella sativa* is a rich source of TQ, *p*-cymene, carvacrol while *V. negundo* consists of viterifolins B and C, and α-selinene. All these chemical constituents are responsible for the treatment of nephrotic syndrome and other kidney-related disorders[19].

Several pharmacological agents are toxic at therapeutic dose and can cause damage to hepatic and renal tissues. With our novel polyherbal formulation, we aimed to prevent the hepatorenal damage of the patients who are likely to take life-saving drugs that also cause the injury to their vital organs such as liver and kidney. Therefore, we intended to assess the combination of these drugs to prevent as well as cure the hepatorenal toxicity induced by CCl_4.

2. Materials and methods

2.1. Chemicals and plant materials

CCl_4 and TQ were purchased from Sigma Aldrich, USA and the standardized ethanolic extracts of the leaves of *B. diffusa*, *S. virgaurea*, and *V. negundo* were procured from Konark Herbal Healthcare, India. Cystone® (*Didymocarpus pedicellata* 130 mg, *Saxifraga ligulata* 98 mg, *Rubia cordifolia* 32 mg, *Cyperus scariosus* 32 mg, *Achyranthes aspera* 32 mg, *Onosma bracteatum* 32 mg, *Vernonia cinerea* 32 mg) was purchased from its manufacturer "Himalaya Drug Company", Bengaluru, India. All the reagents and chemicals used in this study were of analytical grade. The kits used for biochemical tests were Randox kits.

2.2. Preparation of polyherbal formulation

The polyherbal formulation was prepared by mixing the dried standardized ethanolic extracts of the leaves of *B. diffusa*, *S. virgaurea* and *V. negundo* in equal proportions. The dried extract of each drug and TQ (>98% purity) were reconstituted in a proportion of 1:1:1:0.1 to prepare a 100 mg/mL solution in a supersaturated solution of sugar (Modified method adapted from Hussain *et al*.)[20]. The final polyherbal formulation was named as BSVT.

2.3. Animals

Healthy male Wistar rats (weighing 100-200 g) were acclimatized under standard conditions of 12-12 h light and dark cycle at (25±2) ℃ and (55±5)% of relative humidity for the adaptation of laboratory conditions. The rats were fed with standard pellet diet, and water *ad libitum*.

2.4. Experimental design

A total of 36 rats were randomly divided into six different groups (n=6) and housed in standard cages. The rats in each group were given different treatments as per following details. The rats in group 1 were fed with normal saline (0.9%) and served as normal control. Group 2, as CCl_4 control group received CCl_4 which was dissolved in olive oil in a ratio of 1:2 and administered at a dose of 2 mL/kg by intraperitoneal injection, twice a week for four weeks. In group 3, the rats were intoxicated with CCl_4 as same as in group 2 and given Cystone® (dissolved in 10% olive oil) at a dose of 750 mg/kg orally, once daily for four weeks. The rats in group 4-6 were administered with CCl_4 and polyherbal (BVST) treatments orally at a dose of 25, 50 and 100 mg/kg, respectively, once daily for four weeks.

2.5. Serum biochemical analysis

Following the last day of study, blood was collected from the retro-orbital plexus and then all the rats were euthanized to extract the liver and kidneys for histopathological examination. A blood sample for biochemical analysis of serum was extracted from the retro-orbital plexus and was left to stand for half an hour. It was then centrifuged (3 500 rpm for 10 min), and the serum was stored at –20 ℃ for further biochemical analysis. The obtained serum was used to assess the liver function tests including aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), cholesterol, and kidney function tests including blood urea nitrogen (BUN), serum urea concentration, total protein, and serum creatinine. The estimation of all biochemical parameters in serum was carried out by using standard kits[21].

2.6. Evaluation of oxidative stress markers and histopathological analysis

The animals were euthanized by an overdose of chloroform and the liver and kidneys were isolated and washed with normal saline solution (0.9% NaCl solution in distilled water). The organs were then perfused in 50 mmol/L sodium phosphate buffer solution (pH 7.4) which comprised EDTA (0.1 mmol/L) to drain the blood clots and cells. The tissues of both organs were taken and fixed with 10% formalin solution and then sections (4 μm thin) were prepared. These sections were then stained by hematoxylin and eosin for histopathological examination. The microphotographs were taken using a microscope (Leica DM500) connected to Leica ICC50 digital camera at 40 × objective with 50 μm scale bar. The remaining parts of the liver and kidneys were homogenized in phosphate buffer (0.1 M; pH 7.4) and centrifuged at 10 000 rpm for 15 min at 4 ℃. The clear supernatant was then used to assess the antioxidant parameters including superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione peroxidase (GPx).

2.7. Statistical analysis

The analysis was done by one-way analysis of variance, followed by Tukey's test, using Prism 5.0 Graph pad software. Values are expressed as mean ± SD. P value less than 0.05 was considered statistically significant.

2.8. Ethical statement

All animal procedures and experiments were performed by adopting international ethical guidelines of National Institutes of Health on the care and use of laboratory animals. The study protocol was approved by the Institutional Animal Ethical Committee (approval number-SIP/IAEC/PCOL/05/2018).

3. Results

3.1. Effect of BSVT treatment on serum AST, ALT, ALP, and cholesterol

AST, ALT, ALP, and cholesterol levels were significantly increased (P<0.01) in the CCl_4 group compared to the normal control rats, which indicated hepatotoxicity, Cystone®, as a standard drug, recovered these parameters to a normal level. Additionally, the group that received a polyherbal formulation showed dose-dependent amelioration of the renal toxicity (Figure 1).

3.2. Effect of BSVT treatment on serum urea, BUN, total protein and creatinine

The levels of serum urea (68.73 mg/dL), BUN (43.03 mg/dL) and creatinine (2.94 mg/dL) were significantly elevated (P<0.001), while the concentration of total protein was considerably reduced (69.02 g/L) (P<0.001) in the CCl_4 group, indicating the renal toxicity, as compared to the normal control group (Figure 2). Moreover, BSVT treatment recovered these parameters, bringing them near normal in a dose-dependent manner. BSVT at a dose of 100 mg/kg showed the most significant effect, the level of which was similar to that of Cystone® treatment group.

3.3. Effect of BSVT treatment on antioxidant parameters in the liver and kidney tissues

As shown in Table 1, the SOD and GPx activities in the liver tissues of the CCl_4 control group were significantly decreased (P<0.001)

while MDA levels were increased considerably (P<0.001) in comparison to the normal control group. These parameters were recovered in the standard control group treated with Cystone®. In the BSVT treatment group, dose-dependent activity was found in the restoration of the normal levels of MDA, GPx, and SOD. BSVT at a dose of 100 mg/kg showed the best potency in normalizing the disrupted levels of the antioxidant enzymes, and the results were almost similar to that of the standard drug Cystone®.

In Table 2, the levels of SOD and GPx in the kidney tissues were markedly reduced (P<0.001) whereas the level of MDA was raised significantly (P<0.05) as compared to the control animals. The levels of SOD, GPx and MDA were recovered (P<0.001) in the standard drug group and BSVT treatment. The results of BSVT group at a dose of 100 mg/kg were similar to those of the standard drug Cystone®.

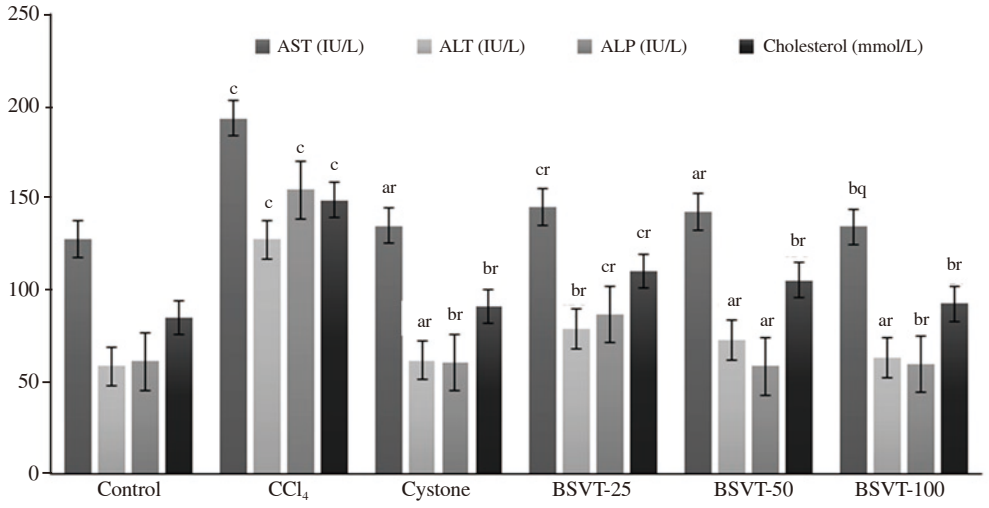

Figure 1. Effect of a novel polyherbal formulation (BSVT) on serum AST, ALT, ALP, and cholesterol. Values are expressed as mean ± SD (n=6). [a]P<0.05, [b]P<0.01, [c]P<0.001 compared to normal controls; [p]P<0.05, [q]P<0.01, [r]P<0.001 compared to the CCl_4 control group. AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alkaline phosphatase.

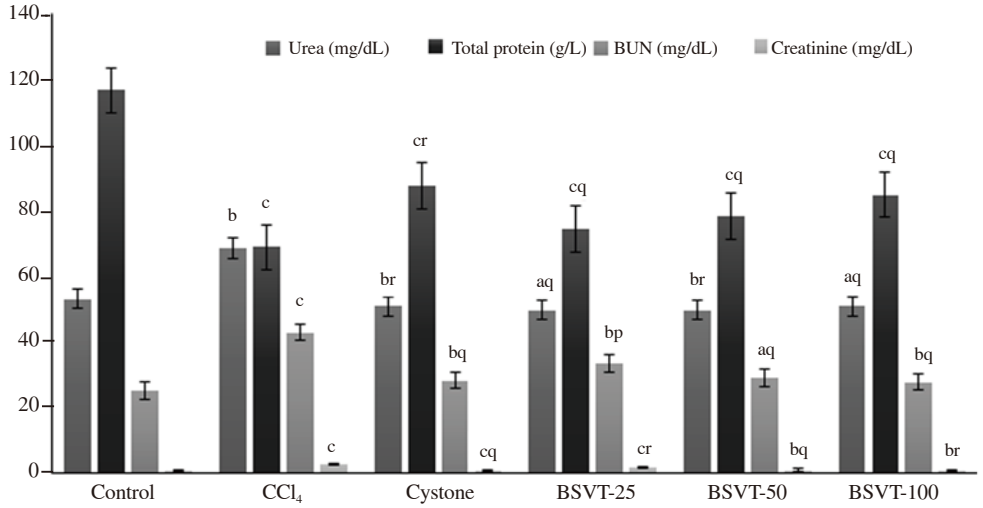

Figure 2. Effect of a novel polyherbal formulation (BSVT) on serum urea, BUN, total protein and creatinine. Values are expressed as mean±SD (n=6). [a]P<0.05, [b]P<0.01, [c]P<0.001 compared to normal controls; [p]P<0.05, [q]P<0.01, [r]P<0.001 compared to the CCl_4 control group. BUN: blood urea nitrogen.

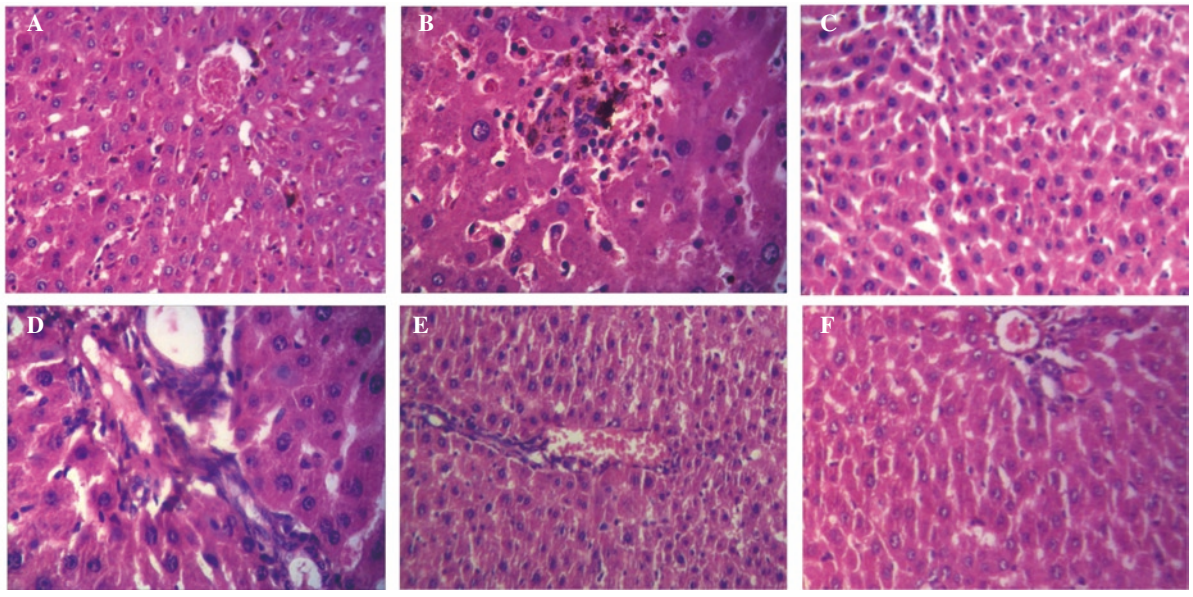

Figure 3. Photomicrographs of liver sections from different groups (H & E, ×40). A: the normal control group shows a normal liver architecture including hepatocytes and central vein (CV) with radiating hepatocytes separated by regular sinusoids. B: the CCl_4 treated group shows damaged hepatic cells with vacuolated cytoplasm, irregular sinusoids and congested CV along with fibrosis and cellular infiltration. C: the Cystone® group shows the restoration of hepatic architecture. However, binucleated cells can be observed. D: BSVT (25 mg/kg) group shows the least alleviation of toxicity. The congestion due to infiltration of the cell in CV is still prominent along with fibrosis in the proximity of CV. Hydropic degeneration and irregular sinusoids are also visible. E: BSVT (50 mg/kg) group shows the hepatic lobules in a nearly hexagonal shape with the CV in the center and abnormal size. The bile duct and the hepatic artery are shown clearly in the picture. F: BSVT (100 mg/kg) treated group shows almost complete restoration of hepatic architecture, CV is distinct and in normal shape, but mild fibrosis can be noticed.

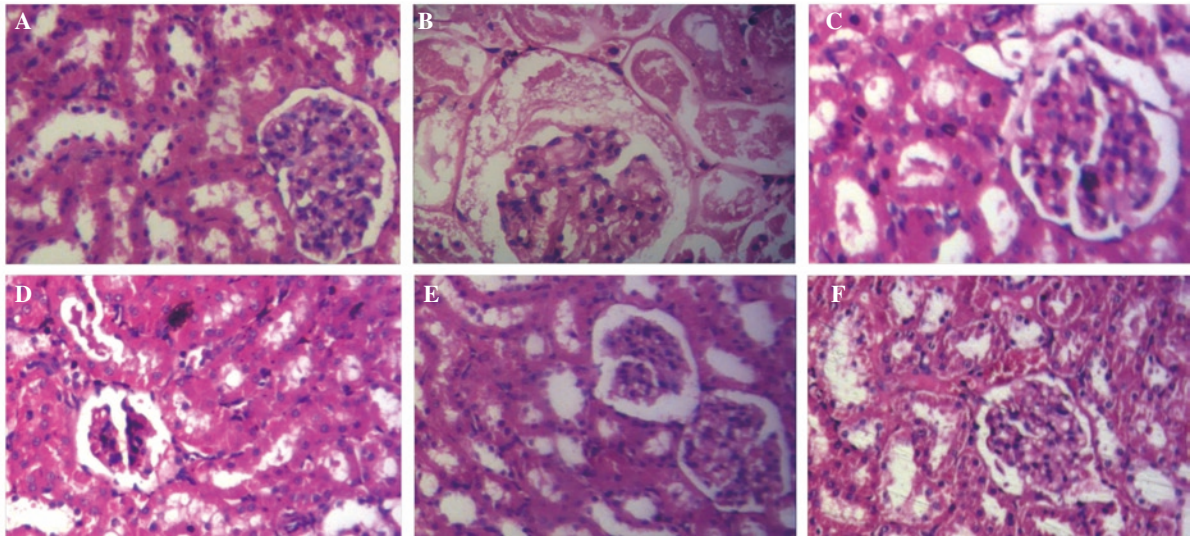

Figure 4. Photomicrographs of renal sections from different studied groups (H & E, ×40). A: the control group shows a normal renal architecture including Bowman's capsule, glomerulus, mesangium, proximal convoluted tubule (PCT) and distal convoluted tubule. B: the CCl_4 treated group shows the loss of brush border on tubular epithelial cells, hydropic degeneration in the tubule epithelium, karyolysis, tubular dilatation, tubular necrosis, epithelial lining necrosis and shrinkage of the Bowman's capsule size. C: the Cystone® treated group shows the restoration of renal architecture. Bowman's capsule is normal in size and PCT regains normal. D: BSVT (25 mg/kg) treated group shows normal PCT with a brush border on tubular epithelium cells and mild karyolysis and tubular necrosis is observed. E: BSVT (50 mg/kg) treated group shows normal PCT with a brush border on tubular epithelium cells with marginal tubular necrosis. F: BSVT (100 mg/kg) treated group shows the restoration of renal architectures, which is strikingly similar to that of the normal control group except for mild epithelial cell necrosis.

Table 1. Effect of a novel polyherbal formulation (BSVT) on oxidative stress markers in the liver.

Groups	MDA (mmol/L)	GPx (ng/mL)	SOD (IU/g)
Control	77.86±2.35	22.58±1.13	20.74±0.66
CCl_4	143.42±3.17^{c}	12.88±0.45^{c}	8.97±0.35^{c}
Cystone®	85.01±3.08^{b,r}	21.85±1.00^{c,r}	18.87±0.76^{c,r}
BSVT-25	93.28±4.72^{c,r}	17.36±0.76^{b,r}	11.45±0.62^{b,r}
BSVT-50	92.50±3.38^{c,r}	18.86±1.12^{c,r}	14.36±0.56^{b,q}
BSVT-100	84.19±2.79^{b,r}	20.79±2.08^{b,r}	19.85±1.45^{b,r}

Values are expressed as mean ± SD (n=6). ^{b}P<0.01, ^{c}P<0.001 compared to normal control; ^{q}P<0.01, ^{r}P<0.001 compared to CCl_4 control group. SOD: superoxide dismutase; MDA: malondialdehyde; GPx: glutathione peroxidase.

Table 2. Effect of a novel polyherbal formulation (BSVT) on oxidative stress markers in the kidney.

Groups	MDA (mmol/L)	GPx (ng/mL)	SOD (IU/g)
Control	68.96±1.41	25.32±0.93	23.60±1.24
CCl_4	139.19±2.77^{c}	16.67±1.11^{b}	11.48±1.13^{c}
Cystone®	76.40±3.11^{b,r}	23.30±1.17^{b,r}	19.82±0.97^{b,r}
BSVT-25	89.90±2.88^{c,r}	20.16±1.55^{b,r}	13.31±0.49^{b,r}
BSVT-50	85.35±3.02^{b,r}	21.46±0.92^{c,r}	18.93±1.16^{b,r}
BSVT-100	74.44±1.93^{b,r}	22.39±1.29^{b,r}	19.41±0.98^{b,r}

Values are expressed as mean ± SD (n=6). ^{b}P<0.01, ^{c}P<0.001 compared to normal control; ^{r}P<0.001 compared to CCl_4 control group. SOD: superoxide dismutase; MDA: malondialdehyde; GPx: glutathione peroxidase.

3.4. Histopathological results

3.4.1. Liver histology

The specimen of the liver section from the normal control group exhibited normal liver architecture. Hepatocytes arranged in cords were radiating out from the central vein and separated by regular sinusoids (Figure 3A). The CCl_4 treated group showed various changes due to hepatic toxicity such as vacuolated cytoplasm indicating hydropic degeneration, irregular sinusoids and congested central vein along with cellular infiltration. Cellular fibrosis was also noted in some of the cells surrounding the central vein. Some of the hepatocytes were also binucleated (Figure 3B). Cystone® treated group showed the restoration of hepatic architecture. Bowman's capsule was normal in size, healthy and distinct glomerulus can be seen with mesangium. Proximal convoluted tubule became normal with brush border, less tubular and epithelial necrosis was seen. Hepatic cells were arranged in a cord pattern radiating from the central vein with proper separation of regular sinusoids, however, the binucleated cell could be seen. Additionally, hydropic degeneration, fibrosis, congestion in the central vein and necrotic cells were absent, indicating the alleviation of hepatotoxicity. But toxicity induced changes such as binucleated cells still could be observed in Cystone® treated group (Figure 3C). The least alleviation of toxicity was observed in 25 mg/kg BSVT treated group, which showed congestion in the central vein due to the infiltration of cells. Moreover, fibrosis was seen around the central vein. There were also hydropic degenerative changes in the hepatocytes, along with irregular sinusoids (Figure 3D). BSVT (50 mg/kg) treated group showed the hepatic lobules in a nearly hexagonal shape with the central vein in the center and abnormal size. The hepatocytes were arranged into cords radiating from the central vein. These hepatocytes were separated by regular blood sinusoids. Bile duct and hepatic artery were shown clearly in the picture. The central vein was not in normal shape (Figure 3E). The treatment groups showed dose-dependent amelioration of the toxicity with maximum normalization in the group treated with BSVT at a dose of 100 mg/kg, which showed the complete restoration of hepatic architecture. The central vein was distinct and in normal shape, along with hepatocytes arranged in the cords radiating from the central vein with regular sinusoids. Nevertheless, mild fibrosis was also noticed in some areas (Figure 3F).

3.4.2. Kidney histology

The histopathological slides of renal sections from the normal control group showed distinct normal renal architecture including Bowman's capsule, glomerulus, mesangium, proximal convoluted tubule and distal convoluted tubule (Figure 4A). Abnormal changes were observed in the CCl_4 treated group such as loss of brush border on tubular epithelium cells, hydropic degeneration in the tubular epithelium, karyolysis, dilatation of the tubules, necrosis of tubular and epithelial lining cells along with the shrinking Bowman's capsule size (Figure 4B). In the standard control group treated with Cystone®, CCl_4 induced toxicity was significantly alleviated such as normal Bowman's capsule, the appearance of brush border on the proximal convoluted tubule and the absence of necrotic cells (Figure 4C). Dose-dependent changes were seen in the treatment groups. BSVT at 25 mg/kg showed normal proximal convoluted tubule with brush border on the epithelial cells, no hydropic degeneration but mild karyolysis and tubular necrosis. Additionally, epithelial lining necrosis and abnormal size of Bowman's capsule were noted (Figure 4D). At a dose of 50 mg/kg, BSVT showed similar results to those of the group treated with 25 mg/kg BSVT, but with better therapeutic changes as only marginal necrosis was observed. Some of the Bowman's capsules seem to be slightly smaller than the normal one (Figure 4E). The results in 100 mg/kg BSVT were strikingly similar to that of the normal control group except for mild epithelial cell necrosis. The treatment showed the restoration of renal architectures with normal Bowman's capsule, healthy and distinct glomerulus and mesangium. Proximal convoluted tubule and distal convoluted tubule regained normal with clear brush border, no tubular necrosis, and mild epithelial necrosis (Figure 4F).

4. Discussion

Carbon tetrachloride is considered as a potential agent for inducing hepatorenal toxicity[22] and is therefore employed in experimental models to induce toxicity in organs like liver and kidney[21,23]. Cytochrome P-450 metabolizes CCl_4 into highly reactive radicals such as CCl_3 (trichloromethyl) and CCl_3O_2 (trichloromethyl

peroxyl) radicals which in turn instigate oxidative changes in both the organs[21,24]. Different studies have observed tubular damage, glomerular damage and necrotic cells in the sections of kidney in animals with CCl_4-induced toxicity[25,26]. In our study, we used the acute intoxication model of CCl_4 to emulate the acute toxicity in humans[22]. As evident, there were several histopathological changes in the sections of the liver and kidney which confirmed the induction of toxicity in both the organs.

ROS are natural substances occurring in every mammalian cell during respiration. Superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2) are the major ROS generated during normal redox reactions in our body[27,28]. Although generated during normal respiration, ROS molecules are cytotoxic in nature. They are generally neutralized by the defensive action of the endogenous antioxidant system, primarily composed of glutathione[27], superoxide dismutase[28], glutathione peroxidase and catalase[29]. The imbalance between the generation and neutralization of ROS can create severe oxidative stress-induced damage, consequently, ROS accumulation may cause protein oxidation leading to the disruption of cell membranes, organelles, and loss of function[30]. In the present study, a significant increase in MDA along with a noticeable decrease in SOD and GPx was observed in the liver and kidney tissues of CCl_4 intoxicated rats, thus showing the occurrence of oxidative stress. It has been known through studies that CCl_4 also attacks mitochondria, apart from GABA receptors in neurons[31], and causes uncoupling of oxidative phosphorylation. This uncoupling interrupts the flow of electron through the electron transport chain and results in severe depletion of adenosine triphosphate, with excessive accumulation of superoxide anion ($O_2^{\bullet-}$) which in turn causes severe oxidative stress[32]. This condition is concurrent with the reduced levels of antioxidants like SOD and GPx, which exacerbates the condition of oxidative stress as the cell is unable to scavenge the excess of $O_2^{\bullet-}$[28]. Excess of $O_2^{\bullet-}$ leads to the frequent generation of H_2O_2, which produces the strongest ROS, *i.e.* $OH^{\bullet}$, by undergoing Fenton reaction[29].

In our study, MDA, which is a marker of lipid peroxidation, was significantly increased in the CCl_4 intoxicated animals, clearly indicating the damage done to the hepatic and renal cells. Cystone® and 100 mg/kg BSVT normalized the levels of SOD, MDA, and GPx. The polyherbal formulation showed its therapeutic effect dose-dependently. Therefore, these results imply that BSVT can reduce ROS in a dose-dependent manner and diminish the oxidative damage in both liver and kidney cells.

Due to the oxidative changes, the cellular membranes of hepatic cells are impaired, consequently leading to the release of hepatic enzymes into the bloodstream[33]. Therefore, serum levels of AST, ALT, and ALP along with cholesterol were elevated significantly in the CCl_4 intoxicated group. ALT is a cytoplasmic enzyme and a relatively specific marker for the necrosis of hepatocytes. The increased level of ALP is attributed to the increased biosynthesis under the rising biliary pressure. Moreover, Sun *et al*. reported that hepatotoxic agents enhance the biosynthesis of adipose cells through the downregulation of AMPKα in 3T3-L1 adipocytes thereby upsetting the metabolism of lipids and this may explain the elevated levels of serum cholesterol in CCl_4 intoxicated animals[34]. Administration of BSVT at 25, 50 and 100 mg/kg significantly prevented hepatotoxicity in a dose-dependent manner. The hepatoprotective effect of different doses of BSVT was exhibited by the decreased serum levels of ALT, AST, ALP, and cholesterol, which may be due to the membrane stabilized by the activity of the polyherbal formulation. The decreased levels of hepatic enzymes imply that BSVT increased the membrane stability and mitigated the intracellular leakage of enzymes as well. The effects of different doses of BSVT were comparable with the standard drug Cystone®. Different doses of BSVT reduced CCl_4 induced elevation in different hepatic enzymes, thereby indicating the protective potential of the novel polyherbal formulation.

Bektur *et al*. revealed in their study that acetaminophen overdose leads to elevated levels of serum BUN and creatinine besides intoxicating the liver[35]. Elevated levels of urea and creatinine in the serum are considered as the index of renal toxicity[36]. In the present study, it was observed that CCl_4 intoxication significantly elevated the BUN, creatinine, and urea but reduced the concentration of total protein, indicating the damage to the liver and renal function. The reduction in the concentration of total protein and an increase in cholesterol levels are ascribed to the early impairment produced in the endoplasmic reticulum which in turn causes a loss in number and function of cytochrome P-450. Therefore, the reduced protein synthesis, increased cholesterol level and the accumulation of triglycerides in the liver cells lead to the fatty liver condition[35]. Oral administration of BSVT significantly attenuated the concentration of BUN, creatinine, and urea, whereas, it increased the serum concentration of total protein. The outcomes of 100 mg/kg BSVT were comparable to that of standard drug Cystone®. The amelioration of these parameters advocates the stabilization of endoplasmic reticulum, which in turn leads to increased protein synthesis and the anti-hyperlipidemic effect of BSVT.

The histopathological observation of our study supplemented the serum biochemical assessments as it showed distinct changes in hepatic and renal architecture due to CCl_4 intoxication. The CCl_4 intoxication caused hydropic degeneration of hepatocytes, congestion, and shrinkage of central vein, fibrosis, cellular infiltration, and irregular sinusoids. All these changes in the CCl_4 group coincided with the findings of Ozturk *et al*. who reported that the administration of CCl_4 is accompanied by hepatic congestion, hemorrhage and necrotic cells[25]. Moreover, according to the report of El-Wessemy *et al*., CCl_4 intoxication caused damage to normal hepatic architecture, congestion in the central veins, and expansion of the portal area accompanied by edema[37]. Our results corroborate the previous finding, and hence, it can be evidenced that hepatotoxicity was induced. These changes disappeared due to the amelioration of the hepatotoxicity by BSVT in a dose-dependent manner. The

ameliorative effect by 100 mg/kg BSVT was in congruence with that of the standard drug group, which can be attributed to the antioxidant activity of BSVT. Moreover, histopathological outcomes of renal damage including tubular dilatation, hydropic degeneration in the tubular epithelium, loss of brush border on tubular epithelium cells, tubular necrosis and shrinkage in the size of Bowman's capsule were observed in the CCl_4 group. Our results were substantiated with the previous finding of Nehru and Anand, who stated that ROS generation and lipid peroxidation are responsible for CCl_4-induced nephrotoxicity[38]. Ogeturk *et al*. revealed a few more changes in the intoxicated renal tissues, such as vacuolated epithelia with foamy appearance[23], dilatation of Bowman's capsule, glomerular atrophy, and inflammatory cell infiltration in the cortical and subcortical areas of the kidney. Our results showed similar results and thus confirmed the induction of renal toxicity in the CCl_4 group. Several studies have asserted that TQ is a potent nephroprotective and hepatoprotective agent. Kanter *et al*., in his study, showed that TQ treatment normalized the renal architecture and restored the cellular organization and function[39]. In addition, other plants used in our novel BSVT formulation are reported to possess potential hepatorenal protective activity. The administration of BSVT ameliorated the toxic condition and significantly reversed the histopathological changes in the renal tissues in a dose-dependent manner, implying that our novel formulation has excellent nephroprotective potential. The previous works support the findings of our research and confirm that BSVT has good potential to reverse both hepatic and renal toxicity.

Several studies have been conducted to assess the potentials of *B. diffusa*, *S. virgaurea*, *V. negundo*, and TQ individually, which have established their nephroprotective and hepatoprotective activity. In our study, we have used all these drugs together to form a polyherbal formulation which proved to be an excellent agent for ameliorating the toxic effects of CCl_4 in hepatorenal cells. Additionally, there are almost no side effects of these herbal drugs and no reports on toxicity due to TQ. Thus, it can be concluded that BSVT is a promising formulation for protecting against hepatorenal toxicity. Clinical studies are still required to prove its efficacy in the future.

Conflict of interest statement

The authors declare no conflict of interest.

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Authors' contributions

AA designed the study, conducted the experiments, performed biochemical investigations, and arranged funding. He had a lead role in drafting the article. MFA played an active role in the arrangement of resources, and served as the project's supervisor. HMA and HIK performed statistical analysis, contributed to the interpretation of the results, and reviewed the final manuscript. NAS interpreted the results and assisted in writing of the manuscript. VA performed the histopathological studies, interpreted the histopathological results and wrote the comments. SS prepared figures and helped in writing of the manuscript. AH contributed to the preparation of polyherbal formulation and critical revision of the article, as well as acted as the project's consultant.

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