doi.org/10.36721/PJPS.2026.39.4.REG.14507.1

Pharmacognostic evaluation and hepatoprotective activity of *Solanum americanum*

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Abstract: **Background**: Solanum americanum, or American black nightshade, is a common weed in Pakistan with a rich history of medicinal applications. Traditionally, its leaves and fruits have been employed to treat various conditions, including skin problems, inflammation, and menstrual irregularities. The plant's therapeutic potential is attributed to its diverse phytochemical composition, encompassing alkaloids, glycosides, and flavonoids. Its traditional use highlights the reliance on folk knowledge for treating liver disease. **Objectives**: This study aimed to investigate the pharmacognostic features and evaluate the hepatoprotective activity of the crude fruit extract of S. americanum against ethanol-induced liver toxicity in rats. **Methods**: An aqueous ethanolic extract of S. americanum fruit was administered to rats with ethanol-induced liver toxicity. Silymarin was used as a reference drug for comparison. Hepatoprotective activity was assessed through biochemical analysis of aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, and total protein levels. Histopathological examination of liver tissues was also conducted. **Results**: The ethanol-treated group exhibited intense hepatocellular injuries and necrosis in liver tissues. Treatment with the aqueous ethanolic extract of S. americanum and silymarin resulted in near-normal lobular architecture, with only slight centrilobular degeneration of hepatocytes and minimal necrotic changes. The S. americanum extract demonstrated hepatoprotective activity, as evidenced by the partial normalization of liver biomarkers. However, its effect was less pronounced than that of silymarin. **Conclusion**: The aqueous ethanolic fruit extract of S. americanum possesses hepatoprotective properties against ethanol-induced liver toxicity in rats. S. americanum mitigates ethanol-induced liver toxicity in rats, partially normalizing liver biomarkers, likely through its antioxidant and nutritional properties, supporting its traditional use in liver disease management.

Keywords: Aqueous ethanolic extract; Biochemical parameters; Hepatoprotective; *S. americanum*; Silymarin; Solanaceae

Submitted on 22-04-2025 – Revised on 04-08-2025 – Accepted on 07-08-2025

INTRODUCTION

S. americanum's therapeutic effects are attributed to its rich profile of bioactive compounds, including steroidal alkaloids (solasodine, solamargine), flavonoids and phenolic acids. These compounds exhibit antioxidant and anti-inflammatory properties, which may mitigate ethanol-induced hepatotoxicity by reducing oxidative damage and stabilizing cell membranes. Critically, UV-Visible (UV-Vis) spectrophotometric (Schemadzu 1900i) analysis of the fruit extract revealed a characteristic absorption peak at 320 nm aligning with the λ_{max} range for flavonoids (320-360 nm) as documented previously (Kumari and Chauhan, 2022). This finding corroborates the plant's high flavonoid content (0.145 ± 0.005 to 0.710 ± 0.036 mg QE/gE), which showed a 4.9-fold dose-dependent increase ($P < 0.05$, ANOVA). Similarly, total phenolics ranged from 0.136 ± 0.002 to 0.645 ± 0.020 µg GAE/mgE, with non-overlapping standard deviations confirming statistical significance (Pandey and Tripathi, 2014). Such quantitative phytochemical profiling, validated via Folin-Ciocalteu and aluminum chloride assays, provides evidence for the observed bioactivity (Singh *et al*., 2022). Despite traditional claims, scientific validation of *S. americanum's* hepatoprotective effects remains limited. Previous studies focused on its antioxidant or anti-inflammatory properties (Alara *et al*. 2021), but this study systematically evaluated its efficacy against ethanol-induced liver injury, a key model for alcoholic hepatitis. Hepatoprotection is evident in ethanol-induced toxicity models, comparing biochemical (AST, ALT, ALP) and histopathological outcomes to silymarin. In addition, the study aimed to standardize *S. americanum* through pharmacognostic evaluation (morphology, microscopy, physicochemical assays). Moreover, the plant's pharmacognostic standardization and dose-response relationships were documented, in order to its integration into evidence-based therapies. Furthermore, quantify bioactive compounds using UV-visible spectrophotometry and fluorescence analysis (Singleton and Rossi, 1965). By bridging ethanol medical knowledge with modern

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analytical and preclinical evidence, this work underscores *S. americanum's* potential as a cost-effective adjunct therapy for liver diseases.

MATERIALS AND METHODS

Chemicals and reagents

Analytical grade acetonitrile, chloroform and hydrochloride were procured from Riedel De Haen (RDH) Germany. Sodium chloride, ferric chloride and formaldehyde were purchased from Merck, Germany. All other chemicals were obtained from Sigma Aldrich (St. Louis, MO, USA) unless otherwise mentioned.

Collection, identification and extraction of plant material

Plant material was collected from the damp, shady areas of various housing societies and local fields in Lahore, Punjab, during the late summer season, and then identified by Dr. Zaheer-ud-Din, Professor of Botany at Government College University, Lahore. The specimen was preserved in the herbarium sheet with a voucher no. GC.HERB.BOT. 3708 at the Department of Botany, Government College University Lahore. After collection of the plant material, it was dried at a temperature not more than 30°C to prevent the decomposition of thermo-labile constituents. *S. americanum*, whole plant material weighing 10 kg, was segregated into leaves, stems and fruits after collection (Elhag *et al*., 2011). Crude extract of dried fruit powder (44.04 g) of *S. americanum* was prepared by cold maceration of coarse powder in hydroalcoholic (70:30) solvent (1:3; 72h, 25°C) (Nyeem *et al*., 2017). The percentage yield of the extract was calculated by the following formula (Azwanida, 2015).

Percentage yield = [(Actual yield/Theoretical yield)] × 100

Macroscopic evaluation

To test various organoleptic characteristics, the fruit part was detached from the plant's other parts (the stem and leaf shoot), manually cleaned and kept over a dry plastic sheet. The scale and magnifying glass were used to measure parameters, including morphology, length and width (Jagtap *et al*., 2016; Kumar *et al*., 2013).

Microscopic evaluation

The small sections of *S. americanum* leaves were studied by using a microtome. The distinguished features of leaf were observed under a microscope with the help of chemical reagents like chloral hydrate, safranine and phloroglucinol (Chanda, 2014).

Physico-chemical investigation

Physicochemical analysis of fruit part was done to evaluate its quality by performing tests like total ash content, water-soluble ash content, acid-insoluble ash content, alcohol soluble ash, sulphated ash, swelling index, foaming index and moisture content which was determined by Loss on drying (LOD) method. Physicochemical analysis was performed by using standard procedures of the World Health Organization (WHO). The values were determined in percentage and compared with the standard values (Organization, 2011).

Phytochemical screening

Phytoconstituents in the hydroalcoholic fruit extract of *S. americanum* were detected by following the standard protocol with little modification (Arora and Sood, 2017; Arora and Onsare, 2014; Savithramma *et al*., 2011; Pradeep *et al*., 2014; Yadav *et al*., 2017; Shaikh and Patil 2020). Alkaloids were detected by four different reagents separately (Dragendorff's, Hager's, Meyer's, Wagner's reagents). The tests were scored positive on the basis of orange to orange-red, turbidity, yellow and brown precipitates, respectively. The presence of purple or violet ring and brick red precipitates respectively in Molisch test, Barfoed's, Benedict's and Fehling's test was considered as positive result for carbohydrates. Proteins were detected by Millon's reagent which changed color form reddish brown. Phenols were detected by ferric chloride solution, with violet coloration as a positive indication. Formation of emulsion and creamy gelatinous precipitate on reaction with 10% sodium hydroxide and lead acetate respectively, is a positive indication for tannins.

Total phenolic content

An aliquot of 90 µL of FC reagent was added to 20 µL (2 mg/mL methanol) of the fruit extract in a 96-well plate. Then 90 µL of Na_2CO_3 was added and incubated for 30 minutes at 37°C. Absorbance was taken at 630 nm using a microplate reader (Elx 800, Biotech, USA). A calibration curve was created using gallic acid (20-100 µg/ml) as a positive control. The phenolic content in the extract was expressed in terms of micrograms of gallic acid equivalent per milligram of crude extract (µg GAE/ mgE) of S. americanum fruit and the experiment was run in triplicate.

Total flavonoid content

Flavonoid contents were determined by aluminum chloride colorimetric method using quercetin as a standard (Ahmed *et al*. 2017). The sample solution approximately 200µL was taken in the test tube, followed by the addition of 1 M potassium acetate (100µL), 5 ml of 10% $AlCl_3$ and 5 ml of DW. The sample was then incubated at room temperature for 45 min. Absorbance was monitored using a UV-Vis spectrophotometer at a wavelength of 415 nm. The flavonoid contents were expressed as mg of quercetin equivalent per g of extract (mg QE/ gE).

Analytical analysis

Extract of *S. americanum* fruit is analyzed UV-visible Spectrophotometer. UV-visible scan of leaf extract of *S. americanum* showed maximum absorption (λmax) at 320nm.

Fluorescence analysis

Phytoconstituents in crude plant extract were analyzed by fluorescence. Fruit powder of *S. americanum* (1 g) were treated with freshly prepared reagents (1 N NaOH, Ammonia, Picric acid, Petroleum ether, 50% HCl, 50% H_2SO_4, Ethyl acetate, Ethyl alcohol, Methanol) and observed in UV lamp light of 254 nm and 365 nm wavelengths (Chanda, 2014).

In-vivo assay

Wistar albino rats (150-300 g, either sex, 6-8 weeks old) were obtained from the animal house of the University of Veterinary and Animal Sciences (UVAS) Lahore, having free access to food and water, and were made habituated to standard laboratory conditions. Rats were kept at a temperature of 26 ± 4°C on a light and dark cycle of 12 hours. All the protocols were followed that were given by the committee and strict compliance was ensured with the principles. There were 6 animals per group in acute toxicity study and determination of hepatoprotective activity (Chauhan *et al*., 2012).

Acute toxicity studies

Acute toxicity studies were carried out normally to evaluate the toxic potential of the plant by the estimation of LD50. Rats were divided into two groups. Group 1, comprised of 6 rats, received the least effective dose of 300mg/kg of body weight, whereas Group 2, comprising the other 6 rats, received a high dose at 2000 mg/kg of body weight. No toxic symptoms were observed during the period except slight dizziness. Moreover, mortality rate was zero percent and which indicated the safe use of plants at considerable doses (Jonsson *et al*., 2013).

Study design for assessment of hepatoprotective activity

The experimental animals were divided randomly into 5 groups. The hepatoprotective activity of plant extracts was determined using the ethanol-induced hepatotoxic model. Group I (Normal control) was given normal saline only, Group II (Disease control) was given 20% ethanol (5.0 g/kg of body weight/day) only, Group III (positive control) rats were administered with standard drug silymarin, Group IV A received a dose of 150 mg and Group IV B received a dose of 300 mg of extract along with 20% ethanol. (Saravanan and Nalini, 2007). The doses were given twice a day as an aqueous solution using an intragastric tube with proper meals and water frequency for 10 consecutive days. In the present study hepatoprotective activity was evaluated biochemically and histopathologically using standard protocol (Ullah *et al*., 2020). Prior to the day of sacrifice, animals were deprived of food overnight, euthanized by the inhalation of chloroform and the blood samples were collected for hematology and serum biochemistry. Blood samples were placed in the EDTA tubes and then centrifuged to obtain serum. Supernatants were decanted and stored at 20 °C for further analysis. Liver was excised, weighed and stored in 4% formalin and then embedded in paraffin and sectioned (4 µm) using a microtome for histopathological study. The samples were dispatched for biochemical screening at University Diagnostic Lab of University of Lahore Teaching Hospital and results had been reported accordingly.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6.01 software (GraphPad Prism software, Inc., CA, USA). The results were expressed as mean ± SD. One-way analysis of variance (ANOVA) followed by Tuckey's test was used to analyze the values. A value of $P<0.05$ was considered significant.

RESULTS

Percentage yield of hydroalcoholic extract of S. americanum fruit

S. americanum fruit extract was prepared in hydro-alcoholic solvent with percentage yield of 14.86% (6.5 g of extract).

Macroscopic examination of S. americanum fruit part

Below is a description of the recorded characters
Shape of fruit: Oval
Dimensions:
Color: Purplish black
Odor: Indistinct
Taste: Bitter
Texture: Smooth
Size: 5-10 mm

Microscopic evaluation of S. americanum leaf part

T.S of leaf through mid-rib explained round to oval cells of upper and lower epidermis were shown in Fig. 1(a), stomatal pore (Fig. 1b), with glandular trichomes and multiple layered parenchyma (Fig. 1c). The cells of parenchyma are thin walled with minute intracellular spaces and vacuole is centrally aligned as shown in Fig. 1 (d).

Physico-chemical evaluation

In physicochemical screening of dried fruit part, water-soluble ash, alcohol soluble ash, sulphated ash and acid-insoluble ash were analysed. Moisture content was within limit of 8-14%. Foaming and swelling index were 115.7 ml and 4.7 ml, respectively. The values are expressed in Table 1.

Phytochemical screening

Different types or concentrations of phytochemicals in extracts are generally regarded as being crucial to any medicinal plant's biological profile (Sarawong *et al*. 2014). The extractive phytoconstituents (Alkaloids, Proteins, carbohydrates, sterols, glycosides, phenols, flavonoids and saponins) in the fruit of *S. americanum* are shown in Table 2. UV-visible Spectrum scan of extract of *S. americanum* is given in Fig. 2.

Total phenolic and flavonoid content

The primary class of secondary metabolites, known as phenolic compounds, has a high link with the anti-inflammatory and antioxidant properties of medicinal plants. As a result, the concentrations of phenolic and flavonoid chemicals in plant extract were determined *in-vitro* in triplicate at different concentrations. Phenolic content determined by Folin-Ciocâlteu reagent and flavonoid content via aluminum chloride method showed increased content with higher concentration. The phenolic and flavonoid content ranged from (0.136 ± 0.002 to 0.645 ± 0.020 µg GAE/mgE) and (0.145 ± 0.005 to 0.710 ± 0.036 mg QE/g), respectively, as shown in Fig. 3.

Fluorescence analysis

Fluorescence analysis of *S. americanum* fruit is expressed in Table 3.

Hepatoprotective activity

The nutritional condition and pathological alterations in the liver can be accurately reflected by the liver index. The body weight of all animals was closely monitored, and high dose of *S. americanum* fruit significantly reduced the liver index. Contrary to this, liver-to-body weight ratio has been shown to be elevated in animals fed with ethanol (Disease control), causing hepatomegaly. Effect of treatment on liver index are shown in Table 4. Liver body- to- weight ratio was found near to normal in animals treated with fruit extract of *S. americanum.* Fig. 4 shows graphically the liver index in *S. americanum* fruit extract.

Hematological changes

Hematological characteristics can be used to explain the effects of a plant extract or its products on the blood, in addition to determining the harmful effects of plant extracts on the blood of animals. Hematological analysis has been performed, and the blood profile has shown modification in the values of blood cells among different treatment groups. Fig. 5 shows the effects of hydro-alcoholic fruit extract of *S. americanum* on the hematological parameters of albino Wistar rats: (A) White Blood Cell (WBC) ($10^3/\mu L^{-1}$); (B) Red Blood Cell **(**RBC) ($10^6/\mu L^{-1}$); (C) Mean Corpuscular Hemoglobin Concentration (MCHC) (g/dl); (D) Hemoglobin (Hb) (g/dl); (E) Mean Corpuscular Volume (MCV); (F) platelet count ($10^3 \cdot$L-1); (G) Neutrophils; (H) Lymphocytes; (I) Eosinophils and (J) Monocytes, during the toxicity study. Table 5 shows the blood profile of various groups of animals administered with the hydro-alcoholic extract of *S. americanum*.

Lipid profile

The values of lipid parameters are shown in Table 6**,** indicating a significant increase in cholesterol, triglycerides (TGs), low density lipoprotein (LDL), very low density lipoprotein (VLDL) but on the other hand high-density lipoprotein (HDL) seems to decline. Fig. 6 shows the lipid profile of animals administered with *S. americanum* extract. Table 6 shows lipid profile of various groups of animals administered with hydro-alcoholic extract of *S. americanum*.

Liver functions

The biochemical analysis of liver was performed and reported to have a significant elevation in the levels of hepatic markers that ultimately account for the malfunctioning of the liver of the rats administered with ethanol. Whereas a noticeable reduction has been found in the experimental models administered with alcoholic fruit extracts of *S. americanum*, restoring them to normal values. These values were comparable to that of standard drug silymarin. The (Table 7 shows the values of ALT, AST, ALP and BILT3 respectively. Fig. 7 shows liver function tests (LFTs) parameters of animals administered with *S. americanum* extract. Table 7 shows LFTs profile of various groups of animals administered with hydro-alcoholic extract of *S. americanum*.

Kidney parameters

The renal parameters give a clear picture of clearance mechanism that has been undergone by kidneys. Slight variations in these biochemical parameters are supposed to be a hallmark of the destruction of renal pathways. There is a marked increase in the values of renal functional markers such as serum creatinine, blood urea nitrogen (Bunchorntavakul and Reddy**,** 2013) and urea showing an obvious decline in kidney function. It has been clearly observed that upon administration of aqueous ethanolic extract of *S. americanum*, these values have been restored to nearly an optimal range, refer to the (Table 8. Fig. 8 shows Serum creatinine levels and Fig. 9 shows renal function tests (RFTs) of animals administered with *S. americanum* extract. Table 8 shows RFTs profile of various groups of animals administered with hydro-alcoholic extract of *S. americanum*.

Quantification of oxidative stress markers

Results showed (Table 9) that MDA levels were within normal range across all groups, indicating controlled lipid peroxidation. Catalase concentrations were elevated in all groups except disease control (2.81 U/mL) and normal control (3.61 U/mL). Both showed normal limits, while other groups like high dose treatment group (4.74 U/mL) and standard (5.32 U/mL**)** showed increased levels. SOD levels were slightly elevated in low dose (8.35%). Elevated values in T2 (48.77%)**,** T3 (33.94%) and especially combination of STD+Ex1 (261.19%)**,** indicating a strong antioxidant response. Normal control had a low SOD (4.01%), close to normal range. Disease control (11.34%) and standard (19.21%) showed moderately elevated SOD.

The treatments, particularly Standard and extract, considerably improved antioxidant enzyme levels (particularly SOD), suggesting a potential protective response against oxidative stress. MDA continued stable, supporting the effectiveness of treatments in controlling lipid peroxidation.

Histopathological analysis with interpretation

Histopathology is the microscopic examination of the diseased tissues. It is a very useful tool for figuring out how diseased tissues look like and what are the characteristic differences between the normal and diseased tissues. Imaging results are shown in Fig. 10 to 15 along with the interpretations when, histopathological examination of hepatocytes is performed.

DISCUSSION

Herbal remedies are in demand worldwide owing to their tremendous benefits and negligible side effects. In this modern era naturopathy has gaining popularity with the growing age, but a major drawback of this disciple is the limitary knowledge in its research and development. Moreover, the quality control parameters of crude drugs are in a developmental stage. Therefore, the WHO has started to make stringent quality principles for phytopharmaceuticals in order to ensure their safety and productivity. It is necessary to standardize herbal extracts through a validated approach and for this purpose, Pharmacognostic evaluation is best possible and reliable method for identifying and evaluating the herbal drugs. Various parameters for pharmacognostic evaluation such as organoleptic features, microscopic studies, phytochemical, physicochemical properties and extractive values determination were carried out on the fruit extract of *S. americanum* in the present study. All these parameters played a pivotal role in the standardization and development validated monograph of this medicinal plant (Mahmood *et al*., 2011).

The study explained high extractive values of *S. americanum* aqueous ethanolic fruit extract yield. This might propose that this extractive yield could be enhanced further by using other extraction methods involve heating such as hot percolation. The extractive value gives an idea for better selection of solvent depending upon the yield of extract in that particular solvent. The extractive value of aqueous ethanolic fruit extract of *S. americanum* was 14.86%. *S. americanum* is an annual or short-lived perennial herb having white or mauve flowers followed by berries. The plant reaches its maximum height at 2m. Berries are oval in shape, purple to black in color, with a glossy texture, varying in size up to 2 mm. Leaves are green, simple alternate, 2.5-8.5 cm long and 2.5 cm wide, toothed, with the base slightly unequal and narrowed at both ends. The transection of leaf has shown upper and lower epidermis, tightly packed with oval to tangentially elongated cells, surrounded by palisade parenchyma and centrally located vascular bundle (Ullah *et al*., 2013).

Physicochemical properties included in the present study were moisture contents, ash values and extractive values. Ash values provide valuable information for determination of quality and purity of crude drugs, particularly when the crude drug is in powdered form. The total ash content is of prime importance especially in the cases where there are chances of adulteration. The presence of different impurities like carbonate, silicate and oxalate could be checked out by these values. The inorganic matter present in the plant material was estimated through water-soluble ash method. The adulteration of this drug with earthy material was assessed by acid- insoluble ash. The total ash, sulphated ash and aci- insoluble ash of *S.americanum* were found to be 12%, 14.87% and 0.5% respectively (Table 1). These values were within the official limits of WHO. The moisture content of *S. americanum* fruit extract was 9.86% which lied in specified limit (Table 1). The decomposition of crude drugs occurs due to the presence of internal moisture which could be a leading cause of microbial contamination resulting in deterioration (Gasti *et al*. 2020).

Preliminary phytochemical screening was a qualitative measure used to ascertain the presence of primary and secondary metabolites in plants and these phytoconstituents impart pharmacological activity to the plant. The results (Table 2) have shown the presence of carbohydrates, Proteins, alkaloids, glycosides, sterols, phenols, flavonoids and saponins in aqueous ethanolic fruit extract of *S. americanum.* Various studies have been reported that the hepatoprotective potential was due to the presence of the glycoalkaloids present in *S. americanum* berries. According to another study, the antioxidant activity of the plant occurred due to the presence of saponins. The present study confirmed the presence of saponins in *S. americanum* by employing the foaming index. The foaming index of *S. americanum* was within the official limits of WHO (Siraj *et al*., 2020). The quantitative estimation of crude *S. americanum* fruit extract was estimated by using total phenolic and flavonoid contents. Phenolic and flavonoid contents are considered as secondary metabolites of plants that play a vital role in defence mechanisms and are directly related to the antioxidant activity of the plant by inhibiting free radicals. The results of total phenolic contents expressed as milligrams of gallic acid equivalents per gram of dry weight are shown in Fig. 3. The comparison between phenolic and flavonoid contents shows that the aqueous ethanolic fruit extract of *S.americanum* is rich in phenolic contents (Veerapagu *et al*., 2018).

S. americanum has shown its biological activity in multiple ways, extending its use to cure inflammation and general illness to an outstanding liver tonic. The Acute toxicity studies that were performed in the study have explained that *S. americanum* fruit extract did not produce any toxic effect, with a zero-death rate reported. Therefore, it is considered safe to be used (Elhag *et al*., 2011). The aqueous ethanolic fruit extract of *S. americanum* also appeared to have hepatoprotective effects against ethanol-induced liver damage. The increased activity of AST, ALT, ALP, and bilirubin concentrations due to the ethanol group was somewhat counteracted by the aqueous ethanolic extract tested in Groups 4 and 5 (Table 7).

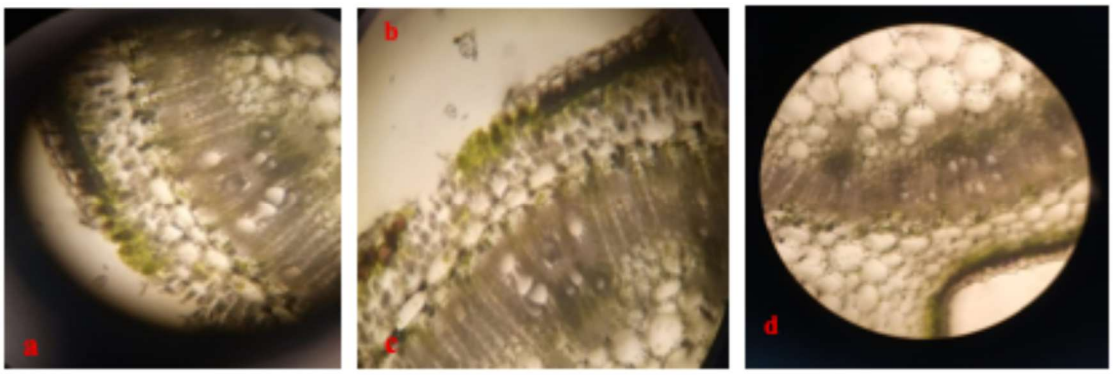

Fig. 1: Microscopic evaluation of *S. americanum* leaves *transversection of leaf showing a: Lower epidermis of Leaf, b: Stomatal pore, c: Palisade mesophyll, d: Vascular bundle*

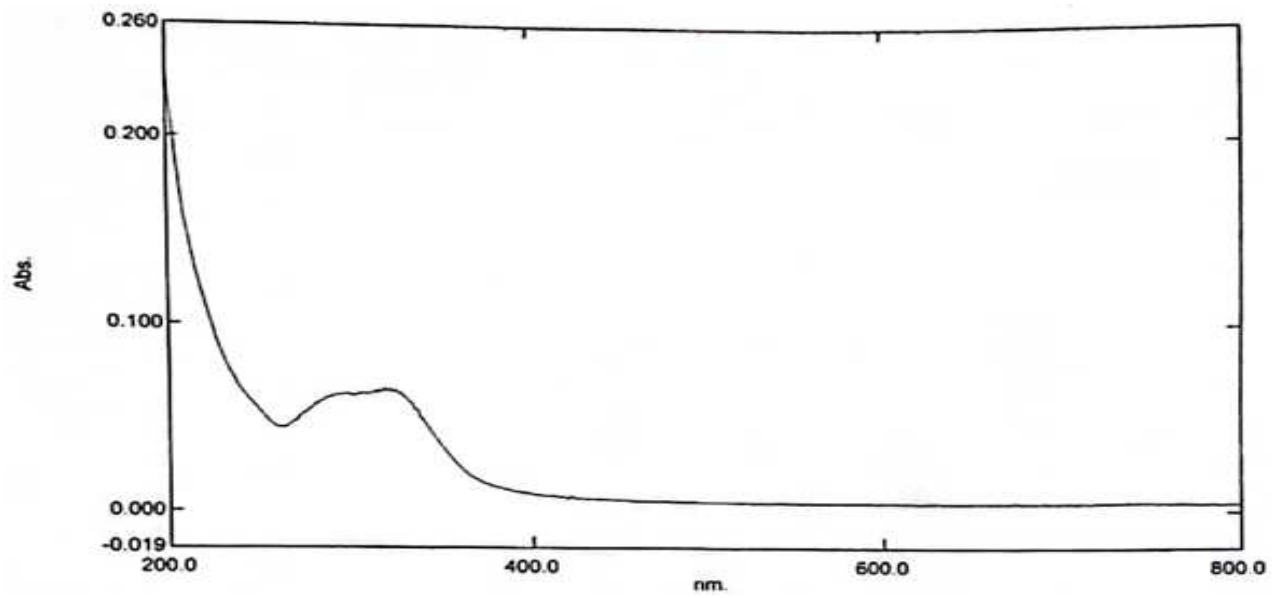

Fig. 2: UV-visible spectrum scan of *S. americanum* fruit extract

Table 1: Physicochemical characteristics of powdered fruit of *S. americanum*

Sr. No.	Physicochemical parameters Parameters	Mean (% w/w) ± SD
1	Total ash	12 ± 0.56
2	water-soluble ash	45.92 ± 0.45
3	Alcohol soluble ash	21.15 ± 035
4	Aci- insoluble ash	0.5 ± 0.61
5	Sulphated ash	14.8 ± 0.32
6	Swelling index	4.7 ± 0.67
7	LOD	9.861 ± 0.24
8	Foaming index	115.7 ± 0.33

Table 2: Phytochemical screening of crude extract of *S. americanum* fruit

Test for identification	Crude extract of *S.americanum* fruit
Alkaloids	
Mayer's test	+
Hager's test	+
Wagner's test	+
Proteins/Amino acids	
Millon's test	+
Carbohydrates	
Benedict's test	+
Fehling's test	+
Sterols	
Salkowaski test	+
Glycosides	
Keller-killiani's test	+
Phenols	
5% $Fecl_3$ solution test	+
Flavonoids	
10% NaOH solution test	+
Saponins	
Foam test	+

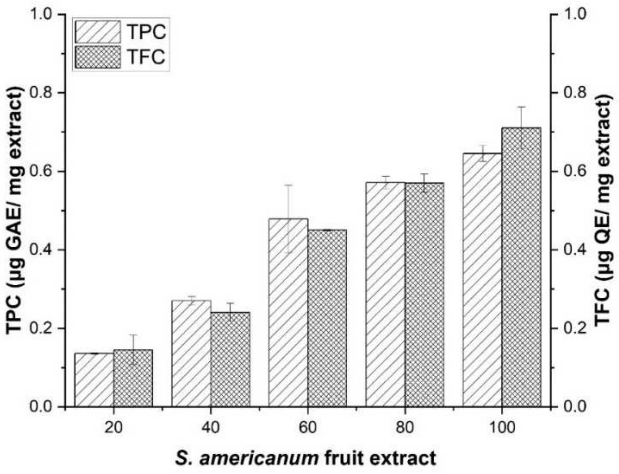

Fig. 3: Total phenolic and flavonoid content in *S. americanum* fruit extract

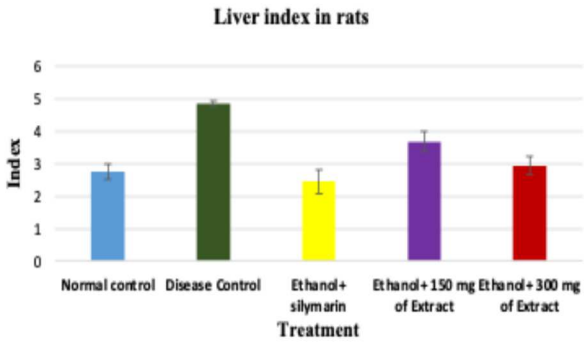

Fig. 4: Liver index in *S. americanum* fruit extract

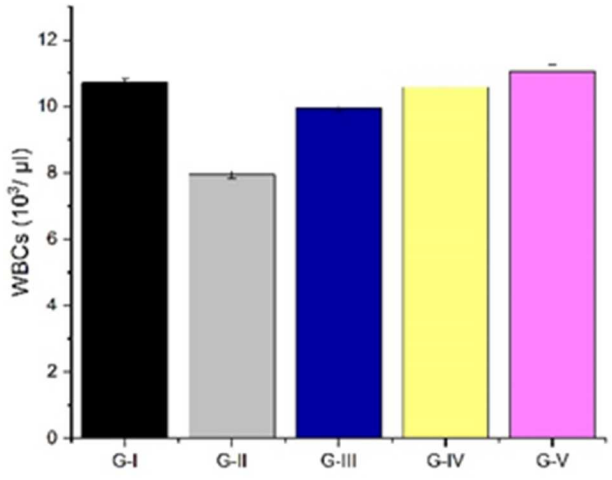

Fig. 5(A)

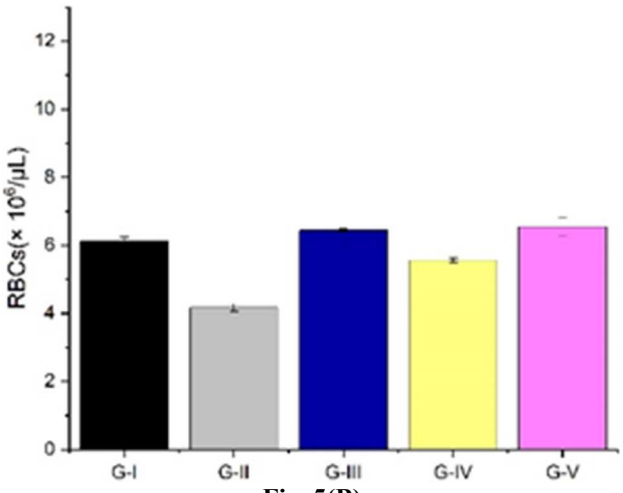

Fig. 5(B)

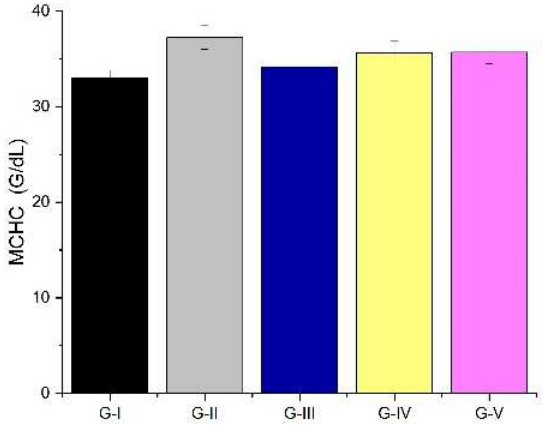

Fig. 5(C)

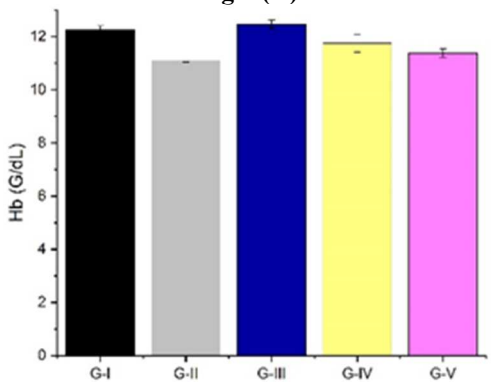

Fig. 5(D)

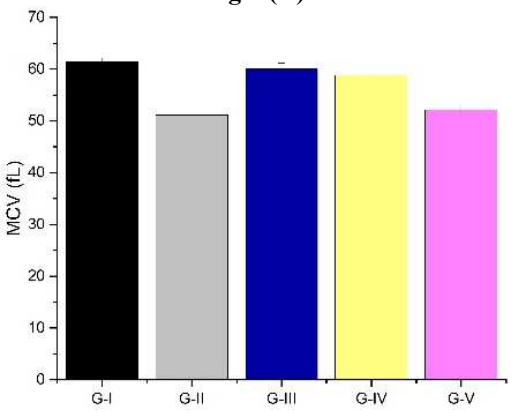

Fig. 5(E)

Elevated levels of ALT, AST, CGT and bilirubin in ethanol-intoxicated rats of group 3 were an indicative factor of necrotic cell damage. Upon administration of Aqueous ethanolic extract of *S. americanum*, the elevated levels of these biomarkers have been restored to nearly normal values as compared to the standard drug silymarin (Bora, 2020). The possible reason behind this reversal would lie in the ability to restore permeability of plasma membrane or regeneration of liver cells (Tufts *et al*., 2015). Repetitive intake of ethanol could be a cause of various physiological changes in the body including slight variation in weight of rats belongs to group 3 (Table 4), but upon supplementation with aqueous ethanolic fruit extract of *S. americanum* an abrupt weight change has been noticed account for its protective effect owes to the nutritional values and detoxifying nature against ethanol-induced toxicity thus restoring the weight loss to normal values.

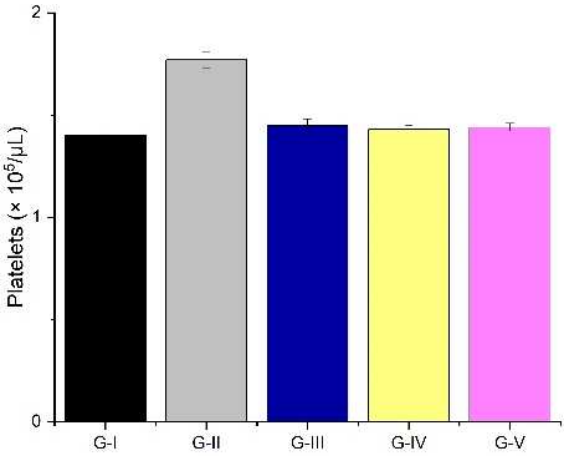

Fig. 5(F)

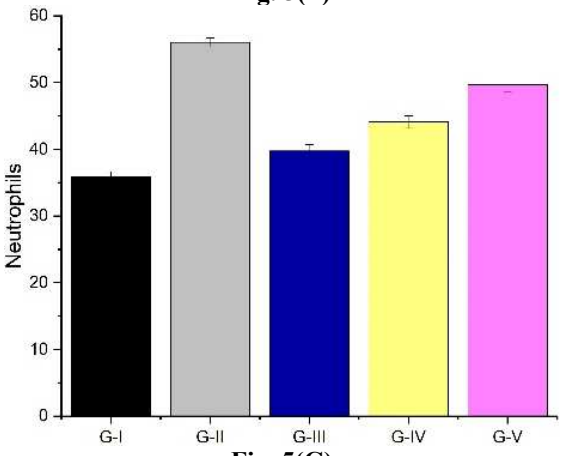

Fig. 5(G)

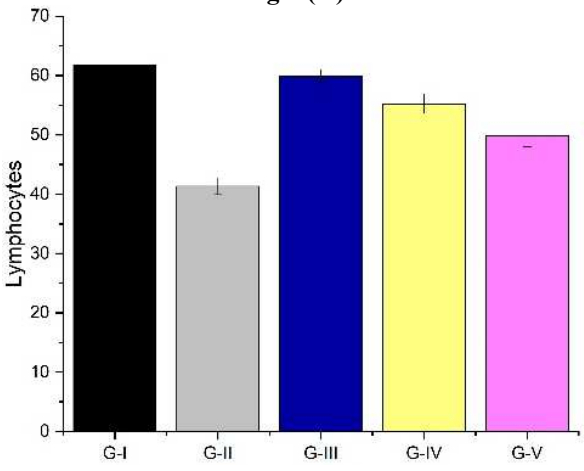

Fig. 5(H)

The hematopoietic system is extremely sensitive to toxins and subsequent ethanolic administration may leads to alterations in the physiological index. Therefore, selected hematological parameters were also analyzed during the study to see the characteristic effect of *S. americanum* fruit extract to normalize the blood profile.

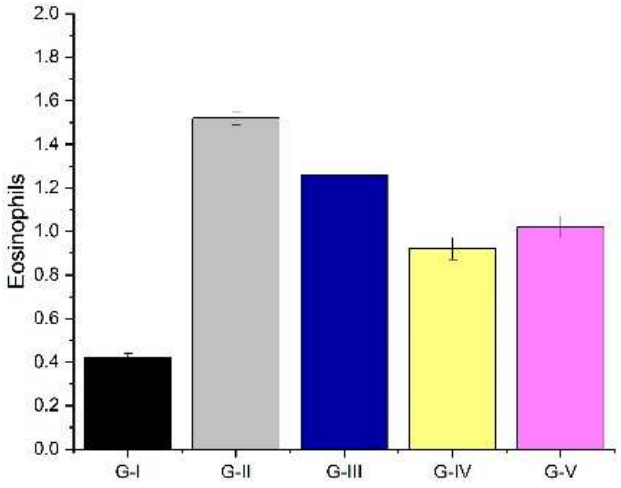

Fig. 5(I)

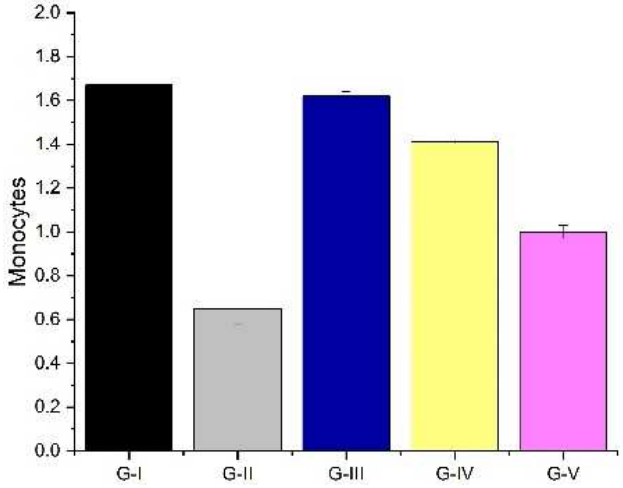

Fig. 5(J)

Fig. 5: Effects of hydro-alcoholic fruit extract of *S. americanum* on the hematological parameters of albino Wistar rats: (A) WBC ($10^3/\mu L^{-1}$); (B) RBC ($10^6/\mu L^{-1}$); (C) MCHC (g/dl); (D) Hb (g/dl); (E) MCV; (F) platelet count ($10^3 \cdot L–1$); (G) Neutrophils; (H) Lymphocytes; (I) Eosinophils and (J) Monocytes, during the toxicity study.

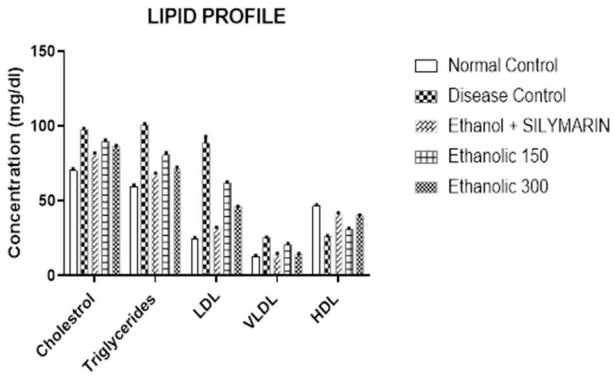

Fig. 6: lipid profile of animals administered with *S. americanum* extract

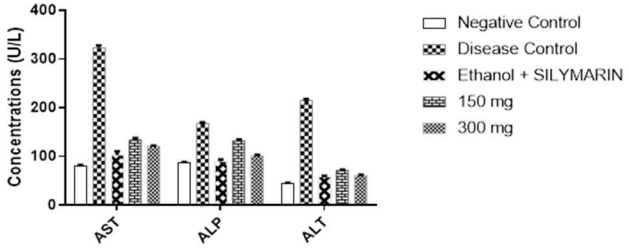

Fig. 7: LFTs Parameters of animals administered with *S. americanum* extract

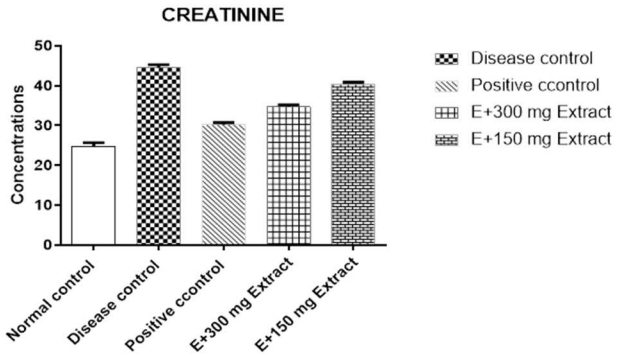

Fig. 8: Serum creatinine levels of animals administered with *S. americanum* extract

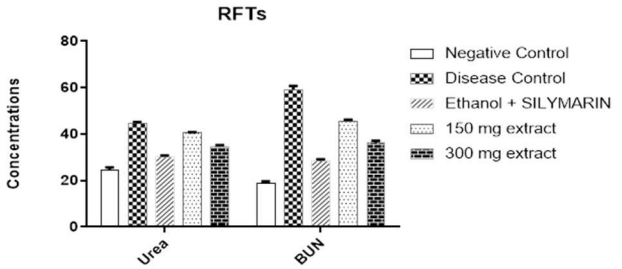

Fig. 9: RFTs parameters of animals administered with *S. americanum* extract

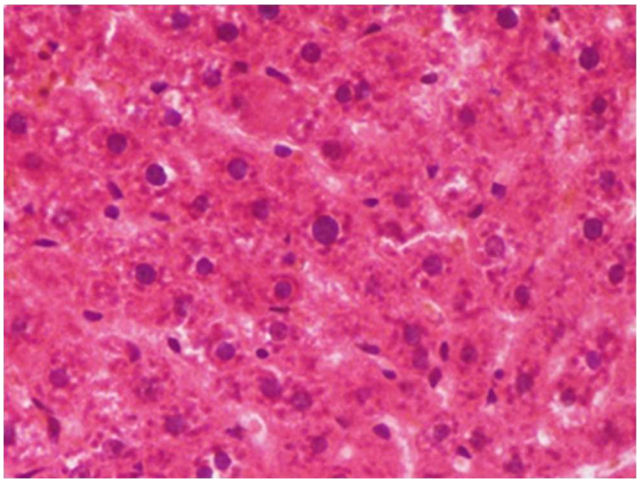

Fig. 10: Normal hepatic parenchymas (NC)

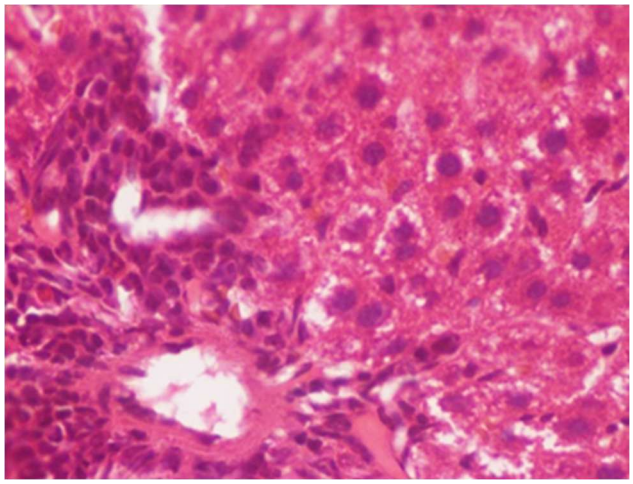

Fig. 11: Central vein accompanying normal hepatocytes (PC)

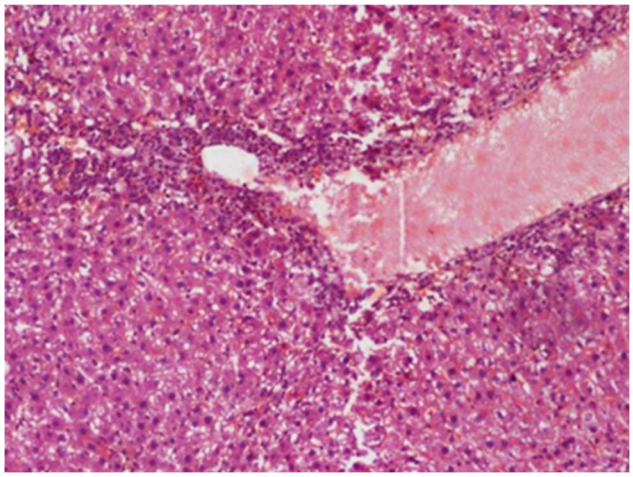

Fig. 12: Hepatocellular degeneration and coagulative necrosis.

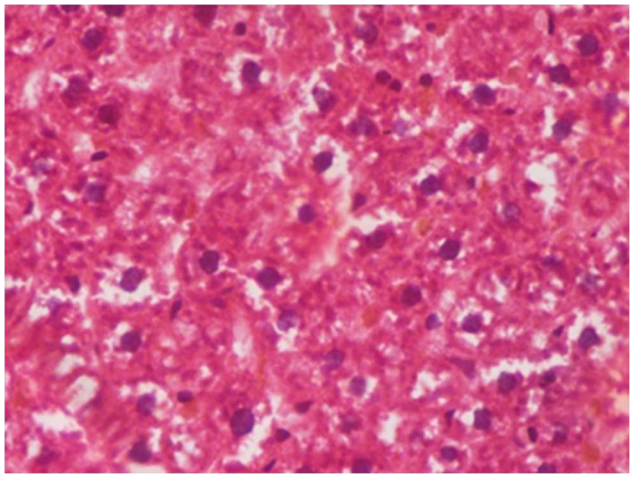

Fig. 13: Shrinkage of sinusoidal spaces with mild degeneration. (Ethanol + Silymarin)

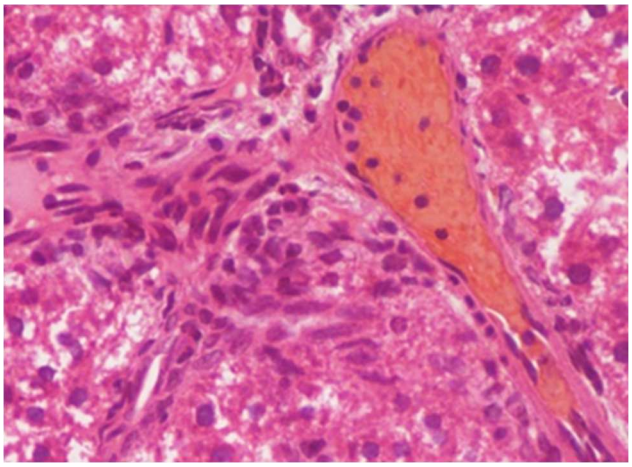

Fig. 14: G-5 infiltration of mononuclear inflammatory cells in the portal area (Ethanol+ 150 mg).

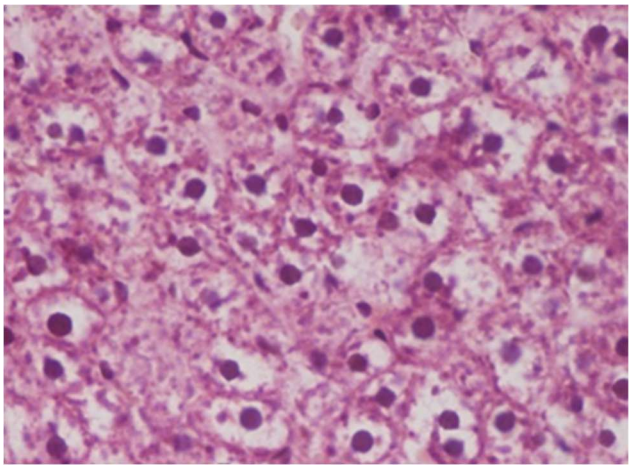

Fig. 15: G-6 there is shrinkage of sinusoidal spaces with mild degeneration (Ethanol+ 300 mg).

The significant reduction in the RBCs and Hb account for decrease in hemosynthesis and erythropoiesis or destruction of RBCs. Hemoglobin act as a free radical and interacts with other drugs or noxious substances, thus leading to membrane peroxidation and hemolysis (Teklehaimanot *et al*., 2015).

Kidneys undoubtedly, play a key role in maintaining homeostasis fluid retention and excretion, the major functional markers of kidneys are urea, uric acid creatinine and Blood urea nitrogen (Bunchorntavakul and Reddy, 2013), raised value of these functional parameters' account for decline in its performance (Table 8). Chronic ethanol consumption may lead to deleterious effect to the organ that has been obvious in the study where the rats administered purely with ethanol show elevated levels of these physiological parameters compared with others receiving aqueous ethanolic fruit extract of *S. americanum*.

Table 3: It shows Flourescence analysis of *S. americanum* fruit

Powdered drug	Visible/Day-light	UV Short (254 nm)	UV Long (365 nm)
Crude drug only	Green	Light brown	Greenish black
Crude drug + Ethanol	Bottle green to dark green	Purple green	Orange red
Crude drug + Methanol	Bottle green	Purple brown	Red
Crude drug + Methylated Spirit	Bottle green	Purple green	Brownish red
Crude drug + Butanol	Yellow green	Light purple	Orange red
Crude drug + Chloroform	Dark green	Chocolate brown	Reddish brown
Crude drug + Toluene	Yellowish green	Yellowish brown	Lemon yellow
Crude drug + Benzene	Brownish green	Brownish green	Orange red
Crude drug + Ethyl acetate	Crystal green	Purple green	Red
Crude drug + Ferric Chloride	Brownish green	Dark brown	Black

Table 4: Effect of *S. americanum.* on body weight, liver weight and liver index.

Groups	Initial body weight (g)	Final body weight (g)	Liver weight (g)	Relative liver wt. (liver weight /100 g of body weight)
Normal control	156 ± 2.18	158 ± 2.23	4.34 ± 0.86	2.76 ± 0.23
Disease control	200 ± 1.92	190 ± 2.17	6.95 ± 1.73	4.87 ± 0.09
Ethanol+ silymarin	220 ± 1.57	255 ± 1.48	4.78 ± 0.90	2.45 ± 0.37
Ethanol+ 150 mg of extract	130 ± 2.23	174 ± 3.20	5.61 ± 1.51	3.68 ± 0.31
Ethanol+ 300 mg of extract	180 ± 2.38	210 ± 2.12	5.76 ± 1.48	2.95 ± 0.27

Table 5: Blood profile of various groups of animals administered with hydro-alcoholic extract of *S. americanum*.

Parameters	Results (mean ± S.D)				
	G-I	G-II	G-III	G-IV	G-V
WBC ($10^3/\mu L$)	10.72 ± 0.12	7.94 ± 0.11	9.94 ± 0.11	10.58 ± 0.37	11.04 ±0.21
RBCs ($\times 10^6/\mu L$)	6.12 ± 0.12	4.17 ± 0.11	6.44 ± 0.04	5.56 ± 0.06	6.54 ± 0.27
Hb (g/dL)	12.26 ± 0.15	11.11 ± 0.07	12.46 ± 0.17	11.75 ± 0.33	11.38 ± 0.16
MCV (fL)	61.34 ± 0.86	51.12 ± 1.69	59.99 ± 1.19	58.79 ± 2.01	52.08 ± 0.91
MCHC (g/dL)	33.01 ± 0.79	37.23 ± 1.24	34.17 ± 2.25	35.63 ± 1.22	35.70 ± 1.23
Platelets ($\times 10^5/\mu L$)	1.40 ± 0.01	1.77 ± 0.44	1.45 ± 0.03	1.43 ± 0.02	1.44 ± 0.02
Neutrophils	35.81 ± 0.81	56.01 ± 0.66	39.73 ± 0.96	44.08 ± 0.92	49.71 ± 1.14
Lymphocytes	61.80 ± 1.25	41.33 ± 1.41	59.92 ± 1.04	55.24 ± 1.64	49.86 ± 1.90
Eosinophils	0.42 ± 0.02	1.52 ± 0.03	1.26 ± 1.79	0.92 ± 0.05	1.02 ± 0.05

Table 6: Lipid profile of various groups of animals administered with hydro-alcoholic fruit extract of *S.americanum.*

Parameters (mg/dl)	Mean±SD				
	G-I	G-III	G-IV	G-V	G-VI
Cholesterol	70.2± 1.30	97.4 ± 1.14	81 ± 1.0	86.2 ± 0.84	89.6 ± 1.14
TGL	59.07 ± 1.47	100.40 ± 1.14	67.40 ± 1.14	71.23 ± 1.27	80.63 ± 1.50
LDL	24.39 ± 1.15	88.57 ± 4.45	31.43 ± 0.84	45.60 ± 0.89	61.79 ± 0.84
VLDL	12.63 ± 0.87	25.09 ± 0.74	13.62 ± 1.33	14.05 ± 0.71	20.71 ± 0.96
HDL	46.58 ± 0.91	26.09 ± 0.57	41.20 ± 0.84	40.03 ± 0.66	31.06 ± 0.60

Table 7: LFTs of various groups of animals administered with hydroalcoholic fruit extract of *S. americanum.*

Parameters (U/L)	G-I	G-II	G-III	G-IV	G-V	Parameters (U/L)
ALT	44.68 ± 1.15	214.35 ± 3.14	57.76 ± 2.18	70.96 ± 1.39	60.79 ± 1.40	ALT
AST	80.37 ± 1.68	323.15 ± 4.62	102.56 ±7.74	133.13 ± 4.58	119.95 ± 2.33	AST
ALP	87.05 ±1.30	167.18 ± 2.81	89.65 ± 3.76	131.78 ± 2.41	101.12 ± 1.88	ALP
TP	7.83 ± 0.17	4.01 ± 0.46	6.66 ± 0.24	4.80 ± 0.43	5.57 ± 0.15	TP
ALB2	3.83 ± 0.14	9.85 ± 0.20	4.19 ± 0.45	7.73 ± 0.34	6.65 ± 0.29	ALB2
CGTS	2.58 ± 0.11	7.83 ±0.14	2.88 ± 0.20	6.27 ± 0.16	5.80 ± 0.23	CGTS
BILT3	0.01 ± 0.01	1.66 ± 0.02	0.10 ± 0.004	0.24 ± 0.008	0.15 ± 0.003	BILT3

Table 8: RFTs of various groups of animals administered with aqueous ethanolic extract of *S. americanum.*

Parameters	Mean ± SD				
	G-I	G-III	G-IV	G-V	G-VI
Urea (mg/dL)	24.71 ± 0.97	44.57 ± 0.69	30.22 ± 0.52	34.71 ± 0.52	40.48 ± 0.42
Creatinine (mg/dL)	0.86 ± 0.01	1.90 ± 0.06	0.86 ± 0.04	0.97 ± 0.06	1.47 ± 0.03
Blood Urea Nitrogen (mg/dL)	18.98 ± 0.73	59.14 ±1.63	28.49 ± 0.69	36.23 ±0.87	45.58 ± 0.63

Table 9: Quantification of oxidative stress markers

Sr. No.	Sample ID	MDA concentration µmol/L Normal range: 1.75 to 9.34	Final value catalase U/mL Normal range: 1.00-3.00 U/mL	SOD final conc. % Normal range: 2.1 to 9.2
1	T1	1.871019108	4.384727871	8.358208955
2	T2	1.616242038	3.832091519	48.7704918
3	T3	1.847133758	4.742324407	33.94833948
4	STD	1.756369427	5.326525771	19.21182266
5	DC	1.590764331	2.816762714	11.34969325
6	NC	1.684713376	3.612092683	4.011461318
7	STD+Ex1	1.708598726	4.03464164	261.1940299

This increase in the values might be due to the increased production of reactive oxygen species and acetaldehyde which act as mediators for tissue damage ultimately leading to renal failure. Thus, *S. americanum* fruit extract play a significant role in retaining the functional capacity of the organ by reducing the impact of ethanol (Chester *et al*. 2019). Thus, from the above-mentioned study, it is evident that *S. americanum* bears a remarkable hepatoprotective role.

CONCLUSION

Our findings demonstrate that *S. americanum* exhibits significant hepato-protective effects against ethanol-induced liver toxicity, as evidenced by the restoration of hepatic biomarkers (AST, ALT, ALP, bilirubin) and histopathological improvements. The aqueous ethanolic extract mitigated ethanol-induced hepatomegaly and oxidative stress, as supported by the modulation of oxidative stress markers (elevated SOD and catalase levels, stable MDA concentrations). These results align with the plant's rich phytochemical profile, including flavonoids and phenolic compounds, which were quantitatively confirmed via spectrophotometric analysis. While the total phenolic (0.136–0.645 µg GAE/mg) and flavonoid (0.145–0.710 mg QE/g) content indirectly suggest antioxidant potential, the observed reduction in lipid peroxidation (stable MDA levels) and upregulation of endogenous antioxidants (SOD, catalase) provide mechanistic support for *S. americanum's* hepato-protective effects. However, further studies including isolation of bioactive constituents are needed to definitively establish the free radical quenching capacity and molecular pathways involved.

In summary, *S. americanum* shows promise as a complementary therapy for ethanol-induced liver injury, likely mediated through a combination of antioxidant, anti-inflammatory and membrane-stabilizing mechanisms. Future research should focus on dose optimization, clinical translation and elucidating structure-activity relationships of its key phyto-constituents.

Acknowledgement
Not applicable

Authors' contributions
Zunaira Nazish has performed the experimental work and written the manuscript. Shoaib Hussain, Rashid Mehmood, Adeel-ur-Rehman, Saeeda Bibi, Ejaz Basheer, Kiran Aslam, Maria Jaleel and Hira Abid have supported the author in performing the experimental work and in writing the initial and final draft of the manuscript.

Funding
There was no funding.

Data availability statement
The authors declare that all the data related to this manuscript is available with corresponding author and corresponding author will share it when required.

Ethical approval
This study has been approved by Animal Ethical Committee of Department of Pharmacy, University of Lahore, Pakistan, wide reference number IREC-2022-24.

Conflict of interest
The authors declare that there are no conflicts of interest, financial or otherwise, related to the publication of this work. No funding or support was received from any organization or entity that could influence the design, execution, or interpretation of this study.

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