A study on efficacy of Shankhpushpi (*Convolvulus prostratus*) Leaf Extract against NSAID Induced hepatotoxicity in Wistar Rats

Km. Jyoti[1], Supriya Singh[2*], Neha jain3, Shailender Mishra[4], Dr. Ram Dayal[5]

Sunderdeep pharmacy college, Sunder Deep Group of Institutions, Ghaziabad, Uttar Pradesh, India.

Corresponding author: Ms. Supriya singh, Assistant Professor, Sunder Deep Pharmacy College, SDGI, Ghaziabad

Background:

Liver diseases, often resulting from drug-induced hepatotoxicity, represent a major global health burden. Non-steroidal anti-inflammatory drugs (NSAIDs), particularly aceclofenac, are known to cause oxidative liver injury. Due to limitations in synthetic hepatoprotective agents, there is growing interest in herbal remedies like *Shankhpushpi* (*Convolvulus prostratus*), a traditional plant with reported anti-inflammatory and nootropic effects.

Objective:

To evaluate the hepatoprotective efficacy of *Shankhpushpi* leaf extract in Wistar rats subjected to aceclofenac-induced liver toxicity.

Methods:

Wistar rats were divided into five groups (n=6 per group), including controls and treatment groups receiving low (200 mg/kg) and high (400 mg/kg) doses of *Shankhpushpi* along with aceclofenac (50 mg/kg). Silymarin (50 mg/kg) was used as a standard reference. The experiment lasted 14 days. Biochemical markers (AST, ALT, ALP, bilirubin, GSH, SOD, and total protein) and histopathological assessments were conducted to evaluate hepatic function.

Results:

High-dose *Shankhpushpi* significantly normalized elevated liver enzymes and improved antioxidant levels, comparable or superior to silymarin. Histopathological analysis showed marked improvement in liver architecture and reduced inflammation in the high-dose group, confirming its protective effect.

Conclusion:

Shankhpushpi leaf extract demonstrated potent, dose-dependent hepatoprotective activity against aceclofenac-induced liver damage. It could serve as a promising natural alternative for managing drug-induced hepatotoxicity.

Keywords: Shankhpushpi, hepatotoxicity, Convolvulus prostratus, aceclofenac, antioxidant, silymarin

Introduction

The high metabolism and excretion take place in the liver, the most common limb in the body. Liver detoxification and the release of several endogenous and exogenous chemicals depend on prevision function; damage or failure in this process can have major health repercussions [1]. Liver injury causes disruption of these metabolic processes. As the body's second largest organ, the liver is responsible for over 5,000 functions, including assisting in blood coagulation, eliminating toxins from the blood, converting food into nutrients, controlling hormones, preventing illness and infection, promoting wound healing, and regulating the levels of iron, glucose, and cholesterol [2,3]. It has a key role in regulating several physiological functions. It also takes part in a number of vital processes, such as storage, secretion, and metabolism. It has a great ability to synthesis advantageous principles and eliminate toxic substances. Lipid peroxidation and other forms of oxidative damage are the principal means by which most hepatotoxins damage liver cells. First synthesized in the twentieth century, paracetamol is a weak analgesic and antipyretic which, when administered in overdoses, causes extensive liver necrosis in man and in experimental animals [4,5].

Oxidative stress is typically associated with hepatic illness. The scientists previously found that aqueous propolis extract (APE) protected hepatocytes from damage caused by carbon tetrachloride (CCl4) in vitro [6]. The current study sought to ascertain if the extract's hepatoprotective qualities might also be applied in vivo [7]. Damage near the liver is linked to changes in these metabolic processes, which disrupts the equilibrium of the organism. However, there is currently no truly adequate medication that is both safe and effective for liver protection in the current medical system [8].

Liver disease and their significance:

Some of these functions are frequently disrupted in conjunction with liver disease. The liver is always in direct contact with a high concentration of harmful substances because blood is delivered to it via the portal vein after intestinal absorption [9]. According to data from the World Health Organization

(WHO), cirrhosis accounts for around 800,000 of the 2.4 million liver disease-related fatalities that occur each year [10].

Even though there are not many synthetic drugs used to treat liver issues, the adverse consequences of their treatment trigger research into alternative therapies such as herbal treatments, which are sometimes inadequate to avoid liver injury caused by drugs [11]. The continued investigation into the synthesis of substances potentially protecting the liver from the harmful effects of pharmaceutical formulations is proof of the necessity for preventive measures [12]. The disadvantages of synthetic drugs make it all the more important to explore natural remedies and the latest liver protection methods to curb the risks. Utilizing the traditional wisdom and developing hepatoprotective drugs based on plants, scientists aim to provide therapeutically neutral and non-toxic means for liver protection [13].

Hepatotoxicity:

Liver failure or damage brought on by taking too many drugs or xenobiotics is the most common symptom of hepatotoxicity [14]. The overuse of pharmaceuticals (acetaminophen, nimesulide, antitubercular treatment approximating isoniazid, rifampicin, etc.) or manufacturing chemicals (alcohol, CCl4, beta galactosamine, thioacetamide, etc.) can result in hepatotoxicants, which are therapeutically relevant exogenous agents that can damage the liver [15]. Hepatotoxicity may lead to liver tumors, cirrhosis, fibrosis, cholestasis steatosis, cytotoxic things (apoptosis, necrosis), and hepatitis [16]. In addition to generalised abdominal discomfort, nausea or vomiting, fatigue, poor energy, delayed bleeding, skin rash, pruritus or broad itching, and pitting edema of the feet, icterus or jaundice, which results in yellowing of the eyes and skin, is one of the symptoms of hepatotoxicity [17].

Acute or chronic inflammatory reactions have been shown to be inhibited by a variety of pharmacological drugs, which may compromise liver function. In a number of liver injury models phosphodiesterases inhibitors have been demonstrated to be protective [18].

Hepato cellular injury and the growth of inflammatory tissue are the results of lipid peroxidation brought on by free radicals [19]. The advantage of this model is that CCl4 can develop fulminant hepatitis, which specifically results in fatty liver and necrosis, in a matter of hours, much like acute hepatitis. A sub lethal concentration of CCl4 will cause irreversible liver damage. At the same time, acute liver injury caused by CCl4 also includes highly regulated phenomena of hepatocyte regeneration following an inflammatory reaction started by local inflammatory cells [20]. Numerous

plants and medicines made from polyherbal ingredients are reported to have hepatoprotective properties. In India, different varieties of medicinal plants are utilized for various combinations to produce proprietary herbal drugs [21]. Liver injury is indicated by reduced tissue GSH concentration, increased tissue lipid peroxidation, and cell necrosis. Furthermore, numerous biochemical indicators, including SGOT, SGPT, triglycerides, cholesterol, bilirubin, and alkaline phosphatase, have increased in serum levels [22].

Anatomy of liver:

A side from everything else, the bile in the liver is essential for digestion. Since they are less costly, more culturally compatible, more body-compatible, and have fewer adverse effects, allopathic drugs are used less often as hepatoprotectives than herbal ones. It has been discovered that these herbal remedies maintain the liver's typical functional state with or without fewer adverse effects [23].

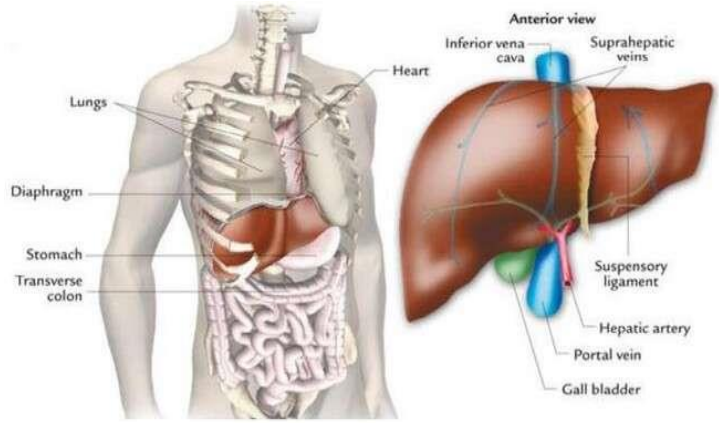


Fig.1.The liver (Howida S, etal, 2016)

Chronic alcoholism, bacteria, and various toxicants, such as carbon tetrachloride, thioacetamide, and several chemotherapeutic medications, have all been shown to cause liver cell destruction [24]. A key element of traditional Indian medicine, encompassing Ayurveda, Siddha, and Unani, is the use of plant-based substances. Since they are less costly, more culturally compatible, more body-compatible, and have fewer adverse effects, herbal drugs are being increasingly used as hepatoprotective treatments compared to allopathic ones [25].

Physiology of liver:

The best ways to classify the liver's numerous functions are as follows: bile secretion, bilirubin metabolism, vascular and haematologic processes, nutrition metabolism, metabolic detoxification, and vitamin and mineral storage [26]. And described the liver's functions in detail as follows:

Bile Sequestration:

Between 700 and 1200 cc of bile are released daily by the liver to aid in intestine digestion. Water, electrolytes, cholesterol, bile salts (conjugated bile acids), and the pigment bilirubin make up bile, an alkaline, yellowish-green, and bitter fluid [27]. Bile is secreted into the canaliculi by hepatocytes. Intestinal absorption and lipid emulsification depend on bile salts or conjugated bile acids. After aiding in the emulsification and absorption of the lipids, the majority of the bile salts are actively absorbed in the terminal ileum. The following is how portal circulation returns them to the liver for resecretion. The acid-dependent fraction and the acid-independent fraction are the two fractionated components of bile [28].

Vascular plus Hematologic Functions: Liver has large capacity to hold much blood to wing to its rich vascular supply. The volume retained at any instant varies in relation to the pressure relationships between veins and arteries [29].

Metabolic Detoxification:

To lessen their toxicity or physiological activity, Hormones, endogenous and exogenous chemicals (including medications), and alien molecules are all altered by the liver [30]. We call this procedure metabolic detoxification. Reducing the excretion of potentially harmful compounds in the intestinal and renal systems, as well as their reabsorption in the intestinal or renal tubules, is known as metabolic detoxification [31].

The Immunologic Function of the Liver: The liver be the key haematopoietic limb during definite phases of foetal life and remains so after birth. All leukocyte lineages can develop from resident haematopoietic stem cells. A variety of haematopoietic cells and haematopoietic stem cells are seen in the liver's portal tract. The liver possesses cells for innate and adaptive immunity. A type of intrinsic immunity as people advance in age, the majority of the cells for innate immunity like monocytes/macrophages and expected killer (NK) cell exhibit compromised purpose [32].

Diagnosis:

Liver Biopsy and Histology:

Alcoholic fatty liver is not always diagnosed by liver biopsy because it is a reversible and non-malignant disorder. To ascertain the extent of the progression of alcoholic liver disease and rule out cirrhosis, a biopsy is carried out in some individuals. Liver biopsies are dangerous operations if coagulopathy and thrombocytopenia are present, even though they are frequently useful in establishing the diagnosis and assessing the degree of liver damage [33].

Laboratory Values and Imaging Studies:

Although certain test abnormalities are diagnostic only, others are of prognostic significance and describe the extent of alcohol-related liver damage. Additionally, the ratio of AST to ALT is often higher; a reversal of this ratio suggests the presence of either concurrent viral hepatitis or nonalcoholic steatohepatitis [34].

Lipid Peroxidation:

Utilizing the spectrophotometric method, lipid peroxidation was quantified in vivo. Malondialdehyde (MDA), the byproduct of lipid peroxidation, was measured using a standard curve that was made by graphing absorbance against the various 1,1,3,3 tetra ethoxy propane (TEP) concentrations, which ranged from 0 to 40 n moles. N moles of MDA/g tissue (wet wt.) is the value that was achieved [35].

Treatment of Hepatotoxicity:

It is most generally referred to as "milk thistle." Silymarin is extracted from Silybummarianum (L.) Gaertn. (Asteraceae/Compositae). One of the earliest and most researched herbs for liver disease treatment is this one. By inhibiting the 5-lipoxygenase pathway, silymarin reduces inflammation by impairing the generation of leukotrienes [36]. According to reports, silymarin shields the liver cells against a variety of poisons, such as D-galactosamine, ethanol, carbon tetrachloride, and acetaminophen. In addition to TNF-induced cytotoxicity, silymarin inhibited the activation of c-Jun N-terminal kinase, caspase, and mitogen-activated protein kinase. When silymarin was administered within 24 hours, serious liver damage (and death) was avoided [37].



Fig.2."Milk Thistle,"

Plant Profile:

Scientific name - Convolvulus prostratus

Kingdom	-	Plantae
Family	-	Convolvulaceae Juss.
Order	-	Solanales
Division	-	Magnoliophyta
Class	-	Magnoliopsida
Sub class	-	Asteridae
Genus	-	Convolvulus L.
Species	-	C.prostratus Forssk



Fig. 3. Convolvulus prostratus.

Medicinal plant of Shankhpushpi (Convolvulus prostratus):

Medicinal plants are regarded as an extremely significant source for medication development since they contain therapeutic compounds. A member of the Convolvulaceae family, Convolvulus prostratus Forssk. (CP), sometimes referred to as "bindweed" or "Shankhpushpi," is a medicinal plant. Numerous advantageous medicinal properties, including analgesic, anti-inflammatory, anti-ulcer, and anti-diabetic actions, are possessed by the plant species Shankhpushpi is a naturally occurring plant in north India that thrives in xerophytic environments [38]. Convolvulus prostratus Forssk. (Convolvulaceae) is characterised by prostrate stems, pale blue blooms, and small oblong, lanceolate, and obtuse leaves. It is expanding rapidly in northwest India [39]. The effects of these herbs on anxiety, depression, and spontaneous locomotor activity were also investigated and contrasted [40].

Materials and methods

Convolvulus prostratus

Shankhpushpi (Convolvulus prostratus) was procured from Royal Green Nursery, Gajrola, District Amroha.

Induction agent: Aceclofenac was used to induce hepatotoxicity in experimental animals.

Dosage: 50 mg/kg body weight, administered orally for inducing liver damage.

Experimental Animals:

Male and female Wistar albino rats, weighing between 180–230 g, were used for the study. The animals were housed in the institutional animal facility under standard laboratory conditions, maintained at a room temperature of 25 ± 2°C with controlled humidity.

Preparation of Materials

- Mature Shankhpushpi plants were identified and collected from the herbal garden.The harvested material was washed thoroughly with tap water to remove surface impurities. After being shade-dried, the fresh leaves were milled into a fine powder. A Soxhlet apparatus was used to treat powdered plant material in order to extract ethanol, while other extraction techniques, such as maceration, may also be applied [41].

- The resulting extract was reduced under diminished pressure and dried afterwards to yield crude ethanolic extract for subsequent analysis.

- **Grouping of Animals:** The rats are split up into five groups, each with six members:

S. No.	Group Name	Description	Dose	Animal Group
1	Control	Distilled water	As per body weight	6
2	Negative Control group)	Aceclofenac	Aceclofenac (50mg/kg,) to induce hepatic damage. Oral per day	6
3	Positive Control group	Aceclofenac + Silymarin	Aceclofenac (50mg/kg,)+ Silymarin (50mg/kg,) oral per day	6
4	Treatment Group 1	Aceclofenac + Shankhpushpi	Aceclofenac (50mg/kg,)+ Shankhpushpi (200mg/kg,) oral per day	6
5	Treatment Group 2	Aceclofenac + Shankhpushpi	Aceclofenac (50mg/kg,) +Shankhpushpi (400mg/kg,). oral per day	6

Parameters assessment:

Biochemical markers: Serum levels of liver enzymes such as:

- **ALT (Alanine aminotransferase)**
- **AST(Aspartate aminotransferase)**
- **ALP(Alkaline phosphatase)**
- **Total bilirubin**

ALT (Alkaline transaminase):

Using a colorimetric method, the aspartate transaminase (AST) and alanine transaminase (ALT) enzyme activity in the serum sample were evaluated. [42].

Procedure:

Reagents 1 (containing LDH, NADH, and α-ketoglutarate) and Reagent 2 were combined to prepare the working reagent. This working reagent was then added to 100 µL of plasma, which had been prepared in Tris buffer (pH 7.5) containing L-alanine. The mixture was thoroughly mixed and incubated for one minute.

After incubation, the change in absorbance was measured at 340 nm for three minutes at regular intervals against a reagent blank. The activity of serum glutamate oxaloacetate transaminase (SGOT/AST) was expressed as International Units per Liter (IU/L) of plasma [43].

ALP (Alkaline Phosphate):

After leaving the blood to coagulate for about half an hour, the serum was spun down by centrifugation at 5000 rpm. It was thereafter stored between 2 and 4 degrees Celsius until analysis was complete [44]. The reaction be initiate by addition 50 µL of the substrate. Alkaline Phosphate test determined the enzyme ALP's activity using the Klaus Walter and Christian Schutt approach (1976). P- nitrophenol and ortho-phosphoric acid are the products of P nitrophenyl phosphate being hydrolysed by ALP [45].

Determination of albumin:

Both globulin and albumin Serum albumin and globulin concentrations were determined using Wotton's method (1964).

Procedure:

To begin, 1 mL of ether was introduced into 5.5 mL of a 28% sodium sulfite (Na_2SO_3) solution, following the addition of 100 µL of serum. To guarantee complete mixing, the mixture was carefully inverted. After that, the test tube was carefully inverted for 20 minutes and safely sealed. Following this incubation time, the sample underwent a 10-minute centrifugation.

After ten minutes, the color's intensity was measured at 540 nm.

Globulin (g/100ml) =Total protein- Albumin [46]

Determination of GSH:

It was determined how much reduced glutathione (GSH) was present in the liver.

Procedure:

5 mL of 10% trichloroacetic acid (TCA) with 10 µL of EDTA and ice-cold phosphate buffer of pH 7.4 was used to homogenize 0.5 g of liver tissue. For maintaining the integrity of the sample, homogenization was carried out at 4 °C. After homogenization, the mixture was centrifuged at 4,000 rpm for 10 minutes at 4°C, and the resulting supernatant was used to measure the concentration of reduced glutathione (GSH) To determine the concentration of reduced glutathione (GSH), 100 µL of the supernatant was mixed with 50 µL of DTNB (5, 5'-dithiobis-(2-nitrobenzoic acid)) and 3.0 mL of 0.2 M hydrochloric acid (HCl) [47].

Determination of SOD activity:

Five micro liters of ice-cold phosphate buffer at pH 7.4 was utilized to homogenize liver tissue samples. Superoxide dismutase (SOD) activity was measured by centrifuging the homogenate for 10 minutes at 4°C at 2,500 rpm. Superoxide dismutase (SOD) activity was determined using the centrifuged supernatant. The tissue homogenate was mixed with 0.1 M Tris buffer and added to the reaction mixture [48].

Result and Discussion

Biomarkers of liver: Effect of Shankhpushpi (Convolvulus prostratus) Leaf Extract on AST/SGOT level in Aceclofenac sodium induced hepatotoxicity in rats.

Table 1. AST data on the means ± SD (n=6)

S.NO.	Treatment group	AST level in liver
1.	Vehicle Control	43.37±2.23
2.	Aceclofenac	118.24±0.79
3.	Standard + Aceclofenac	59.09±3.31
4.	Test Drug low dose + Shankhpushpi	80.43±4.31
5.	Test Drug High dose + Shankhpushpi	61.10±4.26

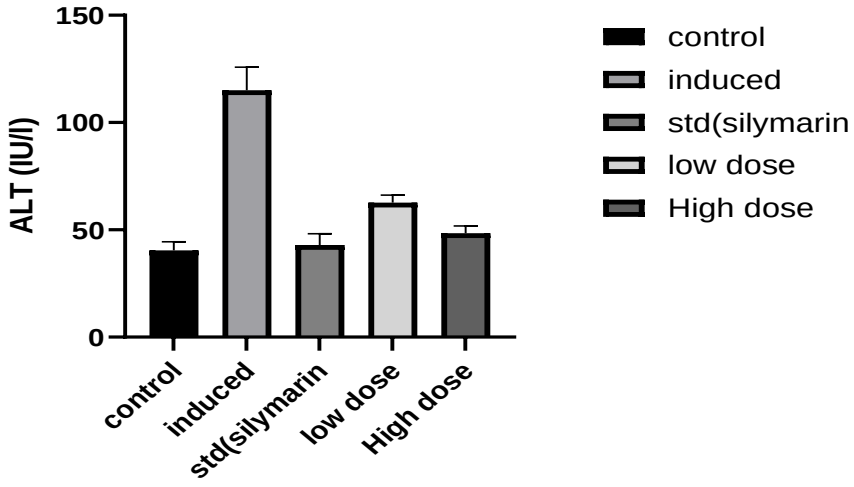


Fig. 4. Graphical representation on ALT

The data represented is mean ± SD, and by using one-way ANOVA, with multiple Turkey's test. Disease control group was significant (P<0.001) v/s normal control, and treatment group (P< 0.001) of Shankhpushpi (Convolvulus prostratus) Test low dose and Test high dose) and Standard group was significant, treatment group (P<0.001) significant against disease group (aceclofenac sodium) (P<0.001) and (Test high dose) treatment group (P<0.001) is highly significant against disease induced group and also highly significant against standard group (P<0.001).

Effect of Shankhpushpi (Convolvulus prostratus) Leaf Extraction ALP level in Aceclofenac sodium induced hepatotoxicity in rats.

Table 2. ALP data on the means ± SD (n=6)

S.NO.	Treatment group	ALP level in liver
1.	Vehicle Control	88.65 ± 9.34
2.	Aceclofenac	197.67 ± 20.36
3.	Standard +Aceclofenac	104.46 ± 14.92
4.	Test Drug low dose+ Shankhpushpi	157.65 ± 10.37
5.	Test Drug High dose+ Shankhpushpi	122.36 ± 5.46

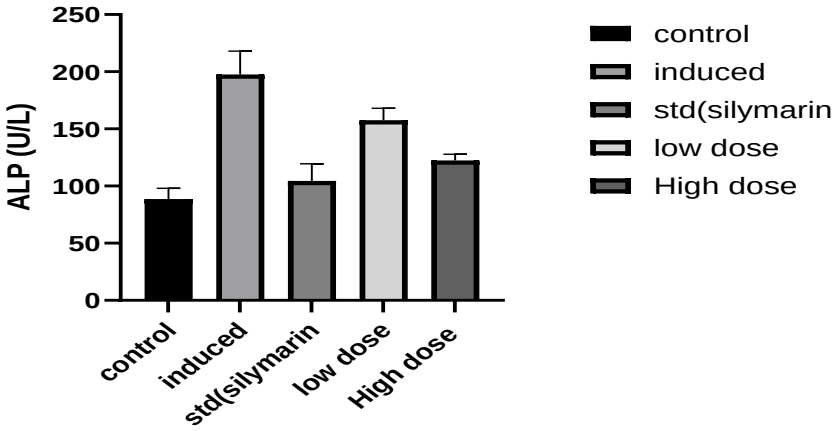


Fig. 5. Graphical representation on ALP

Data given is mean ± SD, and with multiple Turkey's test employing one way ANOVA. Disease control group was significant (P<0.001) v/s normal control, and treatment group (P< 0.001) of Shankhpushpi (Convolvulus prostratus) (Test low dose and Test high dose) and standard group was significant, treatment group (P<0.001) significant against disease group (aceclofenac sodium) (P<0.001), and (Test high dose) treatment group (P<0.001) highly significant against disease induced group, and highly significant against standard group (P<0.001). Effect of Shankhpushpi (Convolvulus prostratus) Leaf Extract on Bilirubin level in Aceclofenac sodium induced hepatotoxicity in rats.

Effect of Shankhpushpi (Convolvulus prostratus) Leaf Extract on Bilirubin level in Aceclofenac sodium induced hepatotoxicity in rats.

Table 3. Bilirubin data on the means ± SD (n=6)

S.NO.	Treatment group	Bilirubin level in liver
1.	Vehicle Control	0.61 ± 0.07
2.	Aceclofenac	1.55 ± 0.23
3.	Standard +Aceclofenac	0.71 ± 0.11
4.	Test Drug low dose+ Shankhpushpi	0.99 ± 0.01
5.	Test Drug High dose+ Shankhpushpi	0.69 ± 0.08

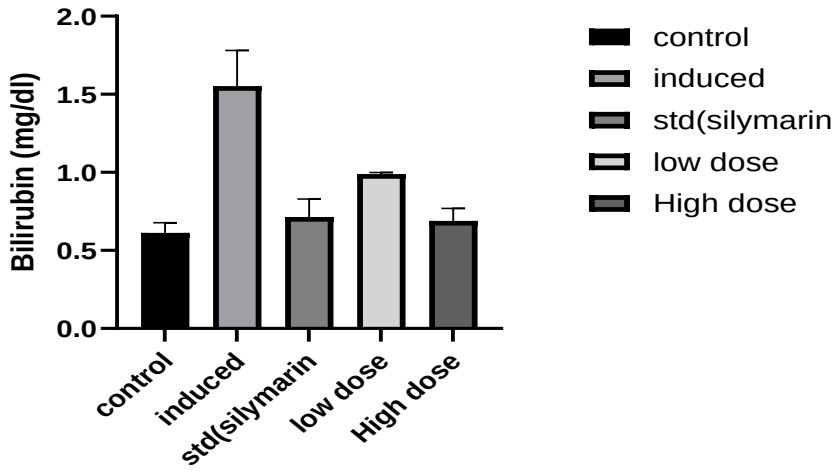
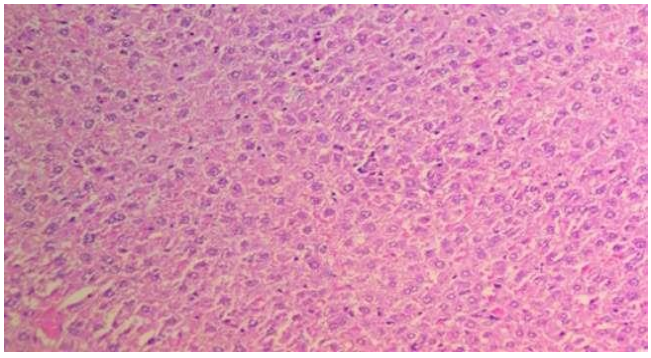


Fig. 6. Graphical representation on Bilirubin

The data represented is mean ± SD and using one-way ANOVA, with multiple Turkey's test. Disease control group was highly major (P<0.001) v/s normal saline control, and treatment group (P<0.001) of Shankhpushpi(Convolvulus prostratus) (Test low dose and Test high dose) and standard group was significant, treatment group (P<0.001) significant against disease group (aceclofenac sodium) (P<0.001) and (Test high dose) treatment group (P<0.001) highly significant against disease induced group and also highly significant against standard group (P<0.001).

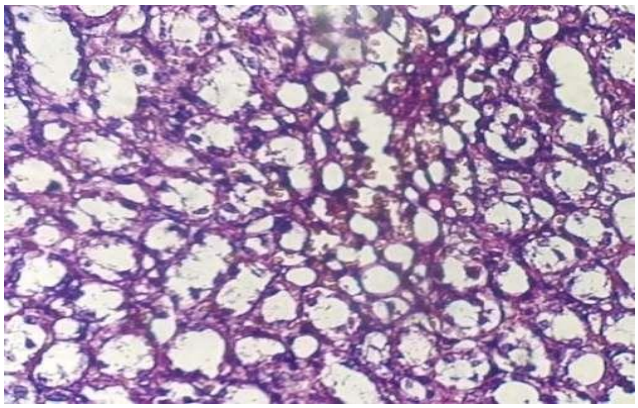
HISTOPATHOLOGICAL ANALYSIS

Group: I (Control Group)



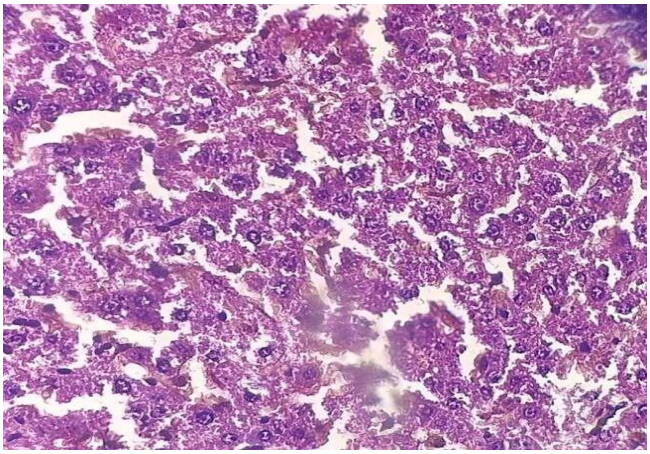
Group: I (Normal Control): Hepatocytes whose cellular structure remains unchanged. No signs of Fibrosis, necrosis, or inflammation. Normal sinusoids gaps and central vein anatomy.

Group: II (Toxicant control)



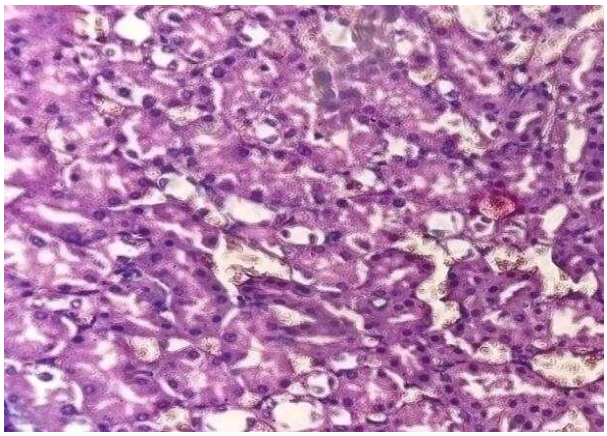
Group: II (Toxicant/Induced control): Hepatotoxicity due to Aceclofenac Sodium –causing extensive liver damage, inflammation, and fibrosis. Hepatocytes ballooning and necrosis. Excessive infiltration of inflammatory cells (Connective Tissue), majorly neutrophils and lymphocytes Extensive fibrosis that can cause nodule formation.

Group: III (Toxicant +Standard)

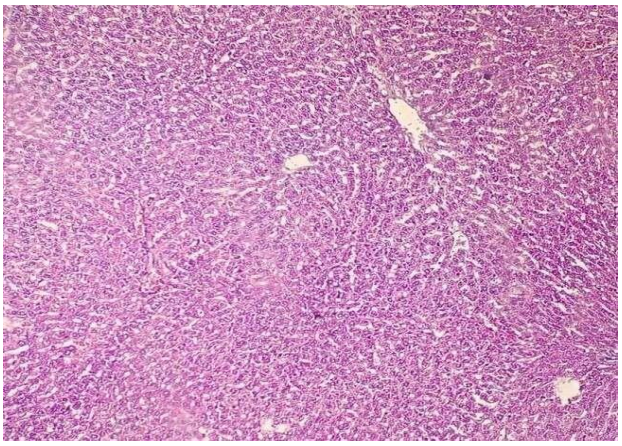


Group: III (Toxicant+ Standard): Decreased liver damage as a result of silymarin's liver-protective properties in comparison to the induced group. Hepatocytes with minimal necrosis or injuries. Less infiltration of inflammation (Connective Tissue).

Group: IV (Toxicant +Low Dose):



Group: IV (Toxicant +Low Dose): Partial protection of the liver by the small dose of Sankhpuspi (convolvulus prostratus) was shown by the moderately decreased liver damage and fibrosis when compared with the induced group. Reduced necrosis and ballooning in the hepatocytes.



Group V (Toxicant+ High Dose): Much reduced liver fibrosis and injury compared to the induced group, demonstrating that the superior dose of Sankpuspi (Convolvulus Prostratus) had significant hepatoprotective activities. Hepatocyte ballooning and necrosis are restricted. Mild inflammation infiltration.

Conclusion

Shankhpushpi (Convolvulus prostratus) can have the strongest hepatoprotective activity based on the overall results of the both biochemical and histological examinations. As indicated in the positive control group Shankhpushpi (Convolvulus prostratus) 400mg/kg significantly normalized the altered conditions induced by aceclofenac sodium such as serum proteins, hepatic enzymes, antioxidant enzymes, bilirubin content, and MDA levels in rats. Combinately, Shankhpushpi (Convolvulus prostratus) showed strong dose-dependent hepatoprotective activities in rats. This assertion is evidenced by blood indicators of liver damage.

References:

1. Li S, Tan HY, Wang N, Cheung F, Hong M, Feng Y. The role of oxidative stress and antioxidants in liver diseases. *Int J Mol Sci*. 2018;19(11):3393. doi:10.3390/ijms19113393.
2. Gao Y, Chu S, Zhang Z, Chen N. Hepatoprotective effects of natural compounds against oxidative stress-induced liver injury: A review. *Front Pharmacol*. 2019;10:620. doi:10.3389/fphar.2019.00620.
3. Wang Y, Zhang X, Li J, et al. Mechanisms of herbal medicines in the treatment of drug-induced liver injury. *Phytomedicine*. 2020;76:153259. doi:10.1016/j.phymed.2020.153259.
4. Abdel-Daim MM, Abushouk AI, Reggi R, et al. Protective effects of silymarin against acetaminophen-induced hepatotoxicity in animal models: A systematic review. *Phytother Res*. 2019;33(7):1693–703. doi:10.1002/ptr.6356.
5. Zhang J, Zhang S, Bi J, et al. Antioxidant and hepatoprotective effects of curcumin against carbon tetrachloride-induced liver injury in rats. *J Funct Foods*. 2018;51:65–71. doi:10.1016/j.jff.2018.10.013.
6. Liu Y, Wang X, Chen Q, et al. Hepatoprotective effects of natural products against non-alcoholic fatty liver disease: A comprehensive review. *Phytomedicine*. 2021;87:153589. doi:10.1016/j.phymed.2021.153589.
7. Sharma P, Kumar A, Sharma S. Herbal medicines for liver diseases: A focus on phytochemistry and pharmacology. *J Ethnopharmacol*. 2020;250:112492. doi:10.1016/j.jep.2019.112492.

8. Xu X, Li W, Zhang L, et al. Hepatoprotective effects of plant-derived polyphenols in drug-induced liver injury. *Oxid Med Cell Longev*. 2020;2020:3420839. doi:10.1155/2020/3420839.
9. Alkandahri MY, Arsyad A, Sari IP, et al. Hepatoprotective activity of medicinal plants: A mini review. *J Pharm Pharmacogn Res*. 2021;9(5):614–27.
10. Chen X, Li D, Sun H, et al. Protective effects of Astragalus polysaccharides on oxidative stress-induced liver injury. *Int J Biol Macromol*. 2020;165:904–12. doi:10.1016/j.ijbiomac.2020.09.227.
11. Salehi B, Vijayakumar V, Sharifi-Rad J, et al. Hepatoprotective effects of herbal medicines: From bench to bedside. *Evid Based Complement Alternat Med*. 2019;2019:5419795. doi:10.1155/2019/5419795.
12. Wang Z, Zhang Y, Liu J, et al. Hepatoprotective mechanisms of flavonoids against oxidative stress and inflammation in liver injury. *Food Chem Toxicol*. 2021;150:112076. doi:10.1016/j.fct.2021.112076.
13. Ahmed SM, Abdel Rahman AA, Mohamed EM. Hepatoprotective potential of *Moringa oleifera* against paracetamol-induced liver injury in rats. *Biomed Pharmacother*. 2019;112:108710. doi:10.1016/j.biopha.2019.108710.
14. Kaur S, Sharma D, Singh AP. Hepatoprotective effects of herbal extracts against carbon tetrachloride-induced liver toxicity in animal models. *J Herb Med*. 2020;22:100351. doi:10.1016/j.hermed.2020.100351.
15. Yang L, Wang Z, Jiang X, et al. Anti-inflammatory and hepatoprotective effects of *Glycyrrhiza glabra* in a rat model of cholestasis. *J Ethnopharmacol*. 2021;271:113896. doi:10.1016/j.jep.2021.113896.
16. Gupta A, Pandey AK, Sharma S. Advances in phytochemicals for the management of liver diseases. *Curr Pharm Biotechnol*. 2020;21(12):1132–45. doi:10.2174/1389201021666200318153836.
17. Madrigal-Santillán E, Madrigal-Bujaidar E, Álvarez-González I, et al. Hepatoprotective effects of natural antioxidants in drug-induced liver injury. *Oxid Med Cell Longev*. 2018;2018:7872439. doi:10.1155/2018/7872439.

18. Zhao H, Zhang Q, Chen S, et al. Hepatoprotective effects of resveratrol in non-alcoholic fatty liver disease. *Nutrients*. 2021;13(7):2432. doi:10.3390/nu13072432.

19. Patel S, Sharma V, Chauhan NS, et al. Hepatoprotective potential of plant-based bioactive compounds: A review. *J Pharm Pharmacol*. 2022;74(2):147–65. doi:10.1111/jphp.13624.

20. Li Y, Xu Y, Li J, et al. Hepatoprotective effects of *Schisandra chinensis* against acetaminophen-induced toxicity in mice. *Phytother Res*. 2019;33(9):2315–24. doi:10.1002/ptr.6419.

21. Abou Seif HS. Physiological changes due to hepatotoxicity and the protective role of some medicinal plants. Beni-suef University journal of basic and applied sciences. 2016 Jun 1;5(2):134-46. [Google Scholar].

22. Adewusi EA, Afolayan AJ. A review of natural products with hepatoprotective activity. J Med Plants Res. 2010 Jul 4;4(13):1318-34.

23. El-Khatib AS, Agha AM, Mahran LG, Khayyal MT. Prophylactic effect of aqueous propolis extract against acute experimental hepatotoxicity in vivo. Zeitschrift für Naturforschung C. 2002 Apr 1;57(3-4):379-85.

24. Ravichandra VD, Ramesh C, Sridhar KA. Hepatoprotective potentials of aqueous extract of Convolvulus pluricaulis against thioacetamide induced liver damage in rats. Biomedicine & Aging Pathology. 2013 Jul 1;3(3):131-5.

25. Delgado-Montemayor C, Cordero-Pérez P, Salazar-Aranda R, Waksman-Minsky N. Models of hepatoprotective activity assessment. Medicina universitaria. 2015 Oct 1;17(69):222-8

26. Rajappa RP, Nallupillai P, Krishna KL, Ramesh MM, Venkatappa AH. Exploring the Mechanisms and Dosages of Herbal Hepatoprotective Agents. Pharmacognosy Research. 2024;16(4).

27. Maqbool M, Rasool S, Dar MA, Bashir R, Khan M. Hepatotoxicity and Hepatoprotective agents: A Mini review. PharmaTutor. 2019 Sep 1;7(9):34-40.

28. Anandan R, Devaki T. Hepatoprotective effect of Picrorrhiza kurroa on tissue defence system in D-galactosamine-induced hepatitis in rats. Fitoterapia. 1999 Feb 1;70(1):54-7.

29. Huang HL, Wang YJ, Zhang QY, Liu B, Wang FY, Li JJ, Zhu RZ. Hepatoprotective effects of baicalein against CCl4-induced acute liver injury in mice. World journal of gastroenterology: WJG. 2012 Dec 7;18(45):6605.

30. Maheswari C, Maryammal R, Venkatanarayanan R. Hepatoprotective activity of Orthosiphon stamineus on liver damage caused by paracetamol in rats. Jordan J Biol Sci. 2008 Sep;1(3):105-8.

31. Kumar A. A review on hepatoprotective herbal drugs. Int J Res Pharm Chem. 2012;2(1):96-102.

32. Ozougwu JC. Physiology of the liver. International Journal of Research in Pharmacy and Biosciences. 2017 Jan 1;4(8):13-24.

33. Kanchana N, Sadiq AM. Hepatoprotective effect of Plumbago zeylanica on paracetamol induced liver toxicity in rats. Int J Pharm Pharm Sci. 2011;3(1):151-4.

34. Yanpallewar SU, Sen S, Tapas S, Kumar M, Raju SS, Acharya SB. Effect of Azadirachta indica on paracetamol-induced hepatic damage in albino rats. Phytomedicine. 2003 Jan 1;10(5):391-6.

35. Scott Luper ND. A review of plants used in the treatment of liver disease: part 1. Alternative medicine review. 1998;3(6):410-21. [Google Scholar]

36. Chouhan G, Solanki N, Shekhawat GS, Parihar S. Convolvulus prostratus Forssk.: A Memory Boosting Herb. Plant Sci. Today. 2023 Jul 11;10(3):86-93.

37. Balachandran I. Phytochemical characterization and evaluation of anti-amnesic activity of two source plants of Shankhapushpi.

38. Malik J, Karan M, Vasisht K. Nootropic, anxiolytic and CNS-depressant studies on different plant sources of shankhpushpi. Pharmaceutical biology. 2011 Dec 1;49(12):1234-42.

39. Dkhil MA, Abdel Moneim AE, Hafez TA, Mubaraki MA, Mohamed WF, Thagfan FA, Al-Quraishy S. Myristica fragrans kernels prevent paracetamol-induced hepatotoxicity by inducing anti-apoptotic genes and Nrf2/HO-1 pathway. International journal of molecular sciences. 2019 Feb 25;20(4):993.

40. Pyati AK, Halappa CK, Pyati SA, Wali V. Serum basal paraoxonase 1 activity as an additional liver function test for the evaluation of patients with chronic hepatitis. Journal of clinical and diagnostic research: JCDR. 2015 Nov 1;9(11):BC12.
41. Hassan SM, El-Kholy WM, Nour SE. Hepatoprotective and antioxidant effects of *Hibiscus sabdariffa* extract against carbon tetrachloride-induced liver injury in rats. *J Food Biochem*. 2020;44(8):e13320. doi:10.1111/jfbc.13320.
42. Khan RA, Khan MR, Ahmed M, et al. Hepatoprotective activity of *Sonchus asper* against carbon tetrachloride-induced liver injury in rats. *Pharm Biol*. 2019;57(1):287–93. doi:10.1080/13880209.2019.1606837.
43. Liu H, Wang C, Zhang Q, et al. Hepatoprotective effects of berberine on liver fibrosis via reducing oxidative stress and inflammation. *Front Pharmacol*. 2020;11:1303. doi:10.3389/fphar.2020.01303.
44. Soni R, Sharma A, Gupta VK. Herbal interventions for drug-induced liver injury: A systematic review. *Phytomedicine*. 2022;103:154246. doi:10.1016/j.phymed.2022.154246.
45. Zhang H, Liu Y, Zhang J, et al. Hepatoprotective effects of green tea extract on oxidative stress-induced liver injury. *Food Sci Hum Wellness*. 2021;10(3):312–19. doi:10.1016/j.fshw.2021.02.015.
46. Ali M, Khan T, Fatima K, et al. Hepatoprotective potential of *Nigella sativa* seeds against acetaminophen-induced liver damage. *J Food Biochem*. 2020;44(10):e13448. doi:10.1111/jfbc.13448.
47. Wang M, Sun J, Jiang Z, et al. Hepatoprotective effects of *Panax ginseng* against drug-induced liver injury: A review. *J Ginseng Res*. 2021;45(4):441–49. doi:10.1016/j.jgr.2020.11.006.
48. Mohamed AA, Elshamy AM, El-Sadek ME. Hepatoprotective effects of *Rosmarinus officinalis* against carbon tetrachloride-induced hepatotoxicity in rats. *J Herbs Spices Med Plants*. 2020;26(4):369–81. doi:10.1080/10496475.2020.1749254.