DEVELOPMENT AND CHARACTERIZATION OF NATURE POLYHERBAL FORMULATIONS FOR THE TREATMENT OF ALCOHOLIC LIVER CIRRHOSIS

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Abstract:

Introduction: Excessive alcohol consumption is a global healthcare problem. The liver sustains the greatest degree of tissue injury by heavy drinking because it is the primary site of ethanol metabolism. Chronic and excessive alcohol consumption produces a wide spectrum of hepatic lesions**.***Salvia miltiorrhiza* Bunge (SM) is a very popular medicinal plant that has been extensively applied for many years to treat various diseases, especially coronary heart diseases and cerebrovascular diseases, either alone or in combination with other Chinese plant-based medicines.*Tinospora cordifolia* commonly named as "Guduchi" is known for its immense application in the treatment of various disease.the plant is of great interest to researchers across the globe because of its reported medicinal properties like anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities.

Objectives: This current research aims to formulate Microencapsulation of polyherbal formulation (PHF) extract was carried. In vivo studies were performed by using Wistar albino rats was screened against paracetamol, carbon tetrachloride (CCl4), and ethanol-induced hepatic damage in rats. PHF was evaluated by measuring levels of serum marker enzymes like SGOT, SGPT, ALP, direct bilirubin (DB), and lactate dehydrogenase (LDH). The histological studies were also studied support the biochemical parameters. Silymarin was used as standard drug

Conclusion: Microencapsulation of polyherbal formulation (PHF) extract of *Salvia miltiorrhiza* and *Tinospora cordifolia* was formulated and results suggests that the hepatoprotective effects of PHF might be useful for liver protection due to combined action of all plant extracts along with their phytoconstituents.

Keywords: Polyherbal formulation, ,*Salvia miltiorrhiza* and *Tinospora cordifolia*,in vivo studies.

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1. Introduction

The alcoholic liver disease covers a spectrum of disorders beginning from the fatty liver, progressing at times to alcoholic hepatitis and culminating in alcoholic cirrhosis, which is the most advanced and irreversible form of liver injury related to the consumption of alcohol [1].Alcohol-related liver disease, including cirrhosis, is a leading cause of death worldwide. In 2016, alcohol was responsible for around 4% of all deaths globally,almost half of all cirrhosis-related deaths in people aged 15 to 49 years worldwide in 2016 were due to alcohol consumption.Alcoholic cirrhosis carries a high risk of mortality, with an overall 5-year survival rate of around 50%.There are three histologic stages of alcoholic liver disease [2].

Alcoholic Fatty Liver or Steatosis - At this stage, fat accumulates in the liver parenchyma.

Alcoholic Hepatitis - Inflammation of liver cells take place at this stage, and the outcome depends on the severity of the damage. Alcohol abstinence, nutritional support, treatment of infection, and prednisolone therapy in severe cases can help in the treatment of alcoholic hepatitis, but more severe cases lead to liver failure.

Alcoholic Cirrhosis - Liver damage at this stage is irreversible and leads to complications of cirrhosis and portal hypertension [3].

Cirrhosis is severe scarring of the liver. This serious condition can be caused by many forms of liver diseases and conditions, such as hepatitis or chronic alcoholism [4].

Cirrhosis often has no symptoms until liver damage is severe. When symptoms do occur, they may include:

- Fatigue.
- Easily bleeding or bruising.
- Loss of appetite.
- Nausea.
- Swelling in the legs, feet or ankles, called edema.
- Weight loss.
- Itchy skin.
- Yellow discoloration in the skin and eyes, called jaundice.
- Fluid accumulation in the abdomen, called ascites (uh-SAHY-teez).

Causes

A wide range of diseases and conditions can damage the liver and lead to cirrhosis.

Some of the causes include:

- Long-term alcohol abuse.
- Ongoing viral hepatitis (hepatitis B, C and D).
- Non-alcoholic fatty liver disease, a condition in which fat accumulates in the liver.
- Hemochromatosis, a condition that causes iron buildup in the body.
- Autoimmune hepatitis, which is a liver disease caused by the body's immune system.
- Destruction of the bile ducts caused by primary biliary cholangitis.
- Hardening and scarring of the bile ducts caused by primary sclerosing cholangitis.

Pathophysiology

Liver cirrhosis occurs when there is ongoing liver damage, usually caused by factors such as chronic alcohol abuse, viral hepatitis, or other causes. This damage leads to inflammation and the development of scar tissue (fibrosis) in the liver. As fibrosis progresses, it disrupts the normal liver architecture, impairs liver function, and increases pressure in the portal vein, resulting in portal hypertension. The liver's ability to filter blood and carry out essential functions becomes compromised, leading to complications such as fluid accumulation in the abdomen (ascites), brain dysfunction (hepatic encephalopathy), and an increased risk of liver cancer. Early diagnosis and management are crucial in slowing down the progression of liver cirrhosis and preventing complications [5].

Treatment

The treatment for liver cirrhosis involves addressing the underlying cause, managing symptoms, and treating complications. If alcohol abuse is the cause, quitting alcohol is crucial. Antiviral medications may be prescribed for viral hepatitis. Medications help manage symptoms, while a healthy diet and vaccinations protect the liver. Complications such as ascites are managed through medication

and procedures, while hepatic encephalopathy is controlled with medications. Variceal bleeding can be prevented or stopped through medications or procedures, and hepatorenal syndrome is treated with specific therapies. Regular monitoring, check-ups, and screenings are important, and in severe cases, liver transplantation may be considered [6]. Overall, treatment aims to slow disease progression, alleviate symptoms, and improve the quality of life for individuals with liver cirrhosis.

Salvia miltiorrhiza

Salvia miltiorrhiza, also known as Danshen or Red Sage, is a traditional Chinese medicinal herb that has been used for centuries belonging to Lamiaceae family. It is a perennial herbaceous plant; it typically grows to a height of 30-60 cm as shown in Figure 1. The plant has an upright growth habit and is characterized by square-shaped stems.

Figure.1. Whole plant of Salvia*miltiorrhiza*

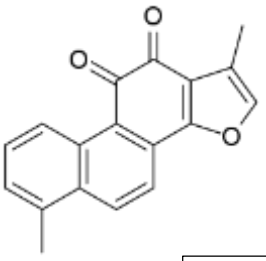
The biological classification of *Salvia miltiorrhiza* is as follows:
Kingdom: Plantae (Plants)
Division: Magnoliophyta (Flowering plants)
Class: Magnoliopsida (Dicotyledons)
Order: Lamiales
Family: Lamiaceae (Mint family)
Genus: Salvia
Species: Salvia miltiorrhiza

Leaves: The leaves of *Salvia miltiorrhiza* are opposite, meaning they grow in pairs on opposite sides of the stem. They have a distinctive heart-shaped or broadly ovate shape, with a pointed tip and a rounded or cordate base. The leaf margin is serrated or toothed, with shallow or deep indentations along the edges [7]. The upper surface of the leaves is typically dark green, while the lower surface may be slightly paler.

Flowers: *Salvia miltiorrhiza* produces small, tubular flowers that are arranged in whorls or clusters along the stem, forming inflorescences. The individual flowers are bilabiate, meaning they have two lips. The corolla, which is the collective term for the petals of a flower, is usually purplish or bluish in color, although variations such as pink or white can occur [8].

Fruit and Seeds: After successful pollination, *Salvia miltiorrhiza* develops fruit in the form of small, dry, brown nutlets. Each nutlet contains a single seed. The nutlets are typically small, measuring around 2-3 mm in length.

Chemical constituents:
The plant contains a diverse array of chemical constituents that contribute to its medicinal properties. These constituents include phenolic acids such as salvianolic acids A, B, C, and D. The plant is also rich in tanshinones, including tanshinone I, tanshinone IIA, and cryptotanshinone, which possess anti-inflammatory, anticancer, and cardiovascular protective properties [9]. Additionally, flavonoids like salvianolic acid B and salvianolic acid A are found. These chemical compounds collectively contribute to the therapeutic potential of in traditional medicine.



Tanshinones

cryptotanshinone

Therapeutic Properties:
Cardiovascular Effects: it is widely used for its cardiovascular benefits. It is believed to improve blood circulation, reduce blood pressure, inhibit platelet aggregation [10].

Anti-inflammatory and Antioxidant Activities: The phenolic acids, tanshinones, and flavonoids exhibit anti-inflammatory and antioxidant effects.

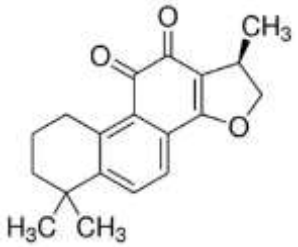
Hepatoprotective Effects:it has been shown to have hepatoprotective properties, protecting the liver against damage induced by toxins or diseases [11].

Anticancer Potential: Some studies have suggested its constituents possess anticancer properties by inhibiting tumor growth, inducing apoptosis (programmed cell death).

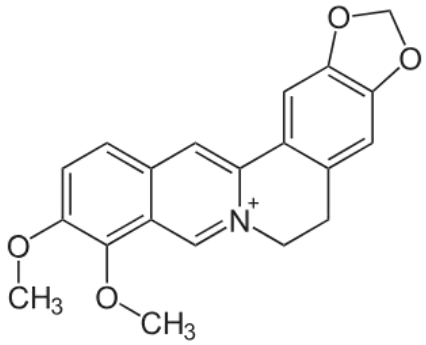
Tinospora cordifolia:
Tinospora cordifolia, commonly known as Guduchi or Giloy, is a medicinal plant native to the Indian subcontinent. It has been used in traditional Ayurvedic medicine for centuries due to its various health benefits.it is a large, climbing shrub as shown in **Figure.2.** that belongs to the family Menispermaceae. It has heart-shaped leaves and aerial roots that help it climb trees or other support structures [12].

Figure.2.Leaves of plant *Tinospora cordifolia*

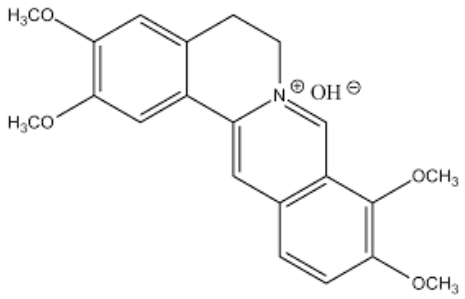
The biological classification of *Tinospora cordifolia* is as follows:
Kingdom: Plantae (Plants)
Division: Magnoliophyta (Flowering plants)
Class: Magnoliopsida (Dicotyledons)
Order: Ranunculales
Family: Menispermaceae
Genus: Tinospora Species: cordifolia



Chemical constituents :
Tinospora cordifolia contains a variety of chemical constituents, including alkaloids such as berberine and palmatine, diterpenoid lactones like tinosporin and columbin, glycosides including cordifolioside A and B, polysaccharides with immunomodulatory properties, steroids such as beta-sitosterol and stigmasterol, and phenolic compounds like catechins and gallic acid [13]. These compounds contribute to the plant's diverse medicinal properties, including antimicrobial, anti-inflammatory, antioxidant, immunomodulatory, and hepatoprotective effects. The specific chemical composition of Tinospora cordifolia can vary depending on factors such as geographical location and plant part used.



Berberine



Palmatine

Medicinal Uses:
Tinospora cordifolia has a long history of use in Ayurvedic medicine for its therapeutic properties. It is considered an adaptogen, which means it helps the body adapt to stress and promotes overall well-being. Some traditional uses include boosting immunity, improving digestion, supporting liver health, reducing inflammation, and promoting general vitality [14]. It is also believed to have antioxidant properties.

In the present experimental study on rats, systematic research was undertaken to evaluate the possible effects of the formulation on the hepatotoxicity induced by diverse agents. The present communication substantiates the therapeutic utility of the formulation as hepatoprotective agents [15].

2. Methodology

2.1 Materials

*Salvia miltiorrhiza*and*Tinosporacordifolia* were purchased from Amsar Private Ltd, Ethanol, olive oil, CCl4, and gum acacia were obtained from LobaChemie, Mumbai. Other drugs and chemicals include paracetamol (Sigma Chemicals), ALT (Alkaline aminotransferase) kit, AST (Aspartate aminotransferase) kit, alkaline phosphate (ALP) kit (Agappe Diagnostics Pvt. Ltd, Ernakulam, Kerla, India), lactate dehydrogenase (LDH) kit (Reckon Diagnostics Pvt. Ltd., India), Bilirubin kit. Remi centrifuge machine, microplate reader (Power wave XS), and micropipette were used.

2.2 Animals

Three-month-old Wistar albino rats of either sex weighing 180–220 g was used for the study. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2° C and relative humidity of 30–70%. A 12:12 light dark cycle was followed. All animals had free access of water [16].

2.2 Phytochemical analysis and HPTLC finger printing

All the plant extracts were identified according to the chemical test and fingerprinting data (Co-TLC with marker). HPTLC is one of the standardization parameters. useful for qualitative and quantitative determination of phytoconstituents present in the herbal extract/ formulation. HPTLC was performed on TLC plate precoated with silica gel 60 GF254 as stationary phase. Various pure solvents of varying polarity were tried in different proportions as mobile phase for the development of chromatogram.

2.3 Preparation of Herbal formulation

The herbal hepatoprotective tablet formulation contains spray dried aqueous extract of*Salvia miltiorrhiza* (150mg) and *Tinosporacordifolia* (200mg)extracts prepared by direct compression technique.

2.4 Acute toxicity study

Swiss albino mice were divided into test group comprising of six animals in each group. The test was performed using increasing oral dose of herbal extracts from 500 to 5000 mg/kg. The mice were observed continuously for 1 h. Hepatoprotective effect of poly herbal formulation and then half hourly for 4 h for any gross behavioural change and general motor activities like writhing, convulsion, response to tail pinching, gnawing, pupil size, faecal output, feeding behavior, etc., and further up to 72 h for any mortality. All the extracts did not cause any significant behavioural changes and no mortality was observed.

2.5Experimental Procedure

2.5.1.Paracetamol induced hepatotoxicity

Healthy albino rats of either sex weighing between 200 and 250 g were provided with rat feed in the same quantity and water ad libitum throughout the experiment. Animals were randomly divided into five groups, six animals. Animal of Group I served as normal received distilled water, Group II was control, received vehicle. Animal of Group III received standard silymarin 50 mg/kg. whereas Groups IV Group V were treated with HHF at 100 and 200 mg/ kg orally for 5 days. On the third day, paracetamol suspension (5% gum acacia) was administered in a dose of 3 g/kg, orally to all groups except normal. Blood was collected after 48 h.

2.5.2 Carbon tetrachloride induced hepatotoxicity

Adult rats of either sex weighing 150–200 g was divided into the five groups of six rats. Group I received distilled water and olive oil 1 ml/kg i.p., Group II served as control received CCl4 1 ml/kg i.p.; Group III received silymarin 50 mg/kg p.o.; while Groups IV and V received 200 and 400 mg/kg HHF orally for 5 days. On third day, hepatotoxicity was induced in all groups by CCl4 with olive oil 1 ml/kg, 1:1, i.p.except normal group. Blood was collected after 48 h.

2.5.3Ethanol-induced hepatotoxicity

Animals were divided into the five groups of six rats. Group I received normal feed and distilled water, Group II served as control, received ethanol (36.6% v/v) 30 ml/ kg/day., in three divided doses. Group III received standard silymarin 50 mg/kg along with ethanol 30 ml/ kg/day, in three divided doses, while Groups IV and V received HHF at the dose of 200 and 400 mg/kg with 36.6% ethanol 30 ml/kg/day in three divided doses for 20 days. All the animals received their respective treatment for 20 days by oral administration. Blood was collected on the 21st day.

2.5.4Assessment of liver function assay

Rats of all groups were anesthetized with ether. Then blood was withdrawn from all groups of rats by puncturing retro-orbital plexus and allowed to coagulate for 45 min at room temperature. Serum was separated by centrifugation (Remi centrifuge machine) at 2500 rpm at 30°C for 15 min. Serum samples were immediately subjected to biochemical estimation of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), ALP, LDH, total bilirubin (TB), and direct bilirubin (DB)

by microplate reader (Power wave XS), according to the colorimetric methods.

2.5.5Histopathological studies

The rats were sacrificed and liver was rapidly excised followed by fixing it for 48 h in 10% formalin, and was dehydrated by passing successively in different mixtures of ethyl alcohol–water (50%, 80%, and 95%) and finally in absolute alcohol, cleared in xylene and embedded in paraffin. Thick sections (4–5 mm) were prepared and then stained with haematoxylin and eosin dye for microscopic observation of cell necrosis, fatty change.

2.5.6Statistical analysis

Results of the biochemical estimations were reported as mean ± S.E.M, and statistical analysis was performed by one-way ANOVA and Dunnet's test. At 95% confidence interval.

3. RESULTS

3.1 Phytochemical analysis

All the extracts showed the presence of glycosides, flavonoids, alkaloids proteins, carbohydrates, and phenolic compounds. According to TLC results, chloroform: methanol (9:1) solvent system for scanned over the wavelength 229 nm, toluene: ethyl acetate: formic acid (5:3.5:1.5) for Phyllanthus at 254 nm, and toluene: ethyl acetate: formic acid (2.5: 5.5: 2 v/v/v) for gallic acid at 254 nm. Results of HPTLC fingerprinting showed the confirmation of Salvia*miltiorrhiza* and *Tinosporacordifolia*of respectively.

3.2 Discussion

Hepatic cells participate in metabolic activities and contain host of enzymes. In tissue, asparate aminotransferase (AST) and alkaline aminotransferase (ALT) were found to be in higher concentrations in cytoplasm, and AST exists in mitochondria. In liver injury, transport function of the hepatocytes gets disturbed, resulting in the leakage of plasma membrane and thereby causing an increased enzyme level in serum The elevated activities of these enzymes are indicative of cellular leakage and the functional integrity of the cell membranes in liver. ALP is excreted by liver via bile in the liver injury due to hepatotoxins, which results in a defective excretion of bile by the liver and is reflected in their increased levels in serum. In drug-induced liver toxicity, the level of LDH, and TB and DB get elevated. The present study was carried out to find out the effect of the formulation on the paracetamol-, CCl4 -, and ethanolinduced hepatotoxicity.

3.3. Paracetamol-induced hepatotoxicity

Paracetamol is a common antipyretic agent which is safe in therapeutic dosage but can produce fatal hepatic necrosis in human, rats, and mice with toxic doses. It is mainly metabolized in liver to excretable glucuronide and sulphate conjugates. Paracetamol is metabolized to a minor electrophilic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI), which during paracetamol overdose depletes glutathione and initiates covalent binding to cellular proteins and initiates cell damageDue to liver injury, the transport function of hepatocytes gets disturbed resulting in the leakage of plasma membrane, thereby causing an increased enzyme level in the serum. In the present study, oral administration of paracetamol (3 g/kg) caused a significant rise in SGPT, SGOT, ALP, LDH, TB, and DB levels. Pretreatment of rats with herbal formulation at a dose of 100 and 200 mg/kg b.w for the 5 days resulted in significant decrease in the level of SGPT, SGOT, ALP, LDH, DB, and TB. as shown in the table 1 Silymarin (reference standard) significantly reduced these levels to normal. The results revealed that hepatoprotective herbal formulation was potent and found to be equipotent with the standard.

Hepatoprotective effect of herbal formulation was further confirmed by the histopathological study of liver as shown in the **Figure 3**. Liver section of paracetamol-treated rats showed gross necrosis of the centrilobular hepatocytes characterized by lymphocytic infiltration. Herbal formulation treated animals show protection against liver damage by minimal necrosis in centrilobular and regeneration of hepatocytes.

Group	Treatment	SGPT (I.U/L)	SGOT (I.U/L)	ALP (I.U/L)	LDH (I.U/L)	TB (% mg)	DB (% mg)
Group I	Normal control (no treatment)	42 ± 4.6	50.3 ± 9.15	212 ± 21	145.7 ± 10.14	0.143 ± 0.01	0.134 ± 0.04
Group II	Toxicant control paracetamol (3 gm/kg)	117 ± 5.9	120 ± 28	319 ± 25	283.4 ± 10.80	0.226 ± 0.01	0.335 ± 0.11
Group III	Silymarin (50 mg/kg) + paracetamol (3 gm/kg)	47 ± 5.2	52 ± 1	165 ± 13	161.2 ± 26.65	0.130 ± 0.01	0.127 ± 0.02*
Group IV	HPF (100 mg/kg) + paracetamol (3 gm/kg)	76 ± 3.0**	75 ± 18	220 ± 6.5	188.5 ± 6.24*	0.130 ± 0.01	0.142 ± 0.02
Group V	HPF (200 mg/kg) + paracetamol (3 gm/kg)	62 ± 7.65	62 ± 12	180 ± 10**	167.8 ± 37.6	0.147 ± 0.02	0.124 ± 0.03*

Table.1. Effect of polyherbal formulation and silymarin on serum parameters in paracetamol-induced hepatic damage in rats.

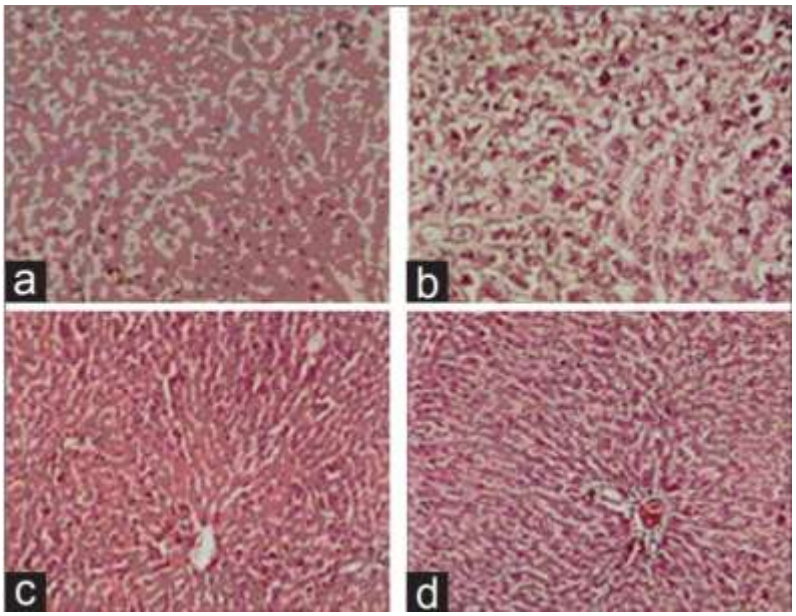


Figure.2. Histopathology of liver tissues. Histopathological study of paracetamol-induced hepatotoxicity

3.4Carbon tetrachloride induced hepatotoxicity

It is well established that hepatotoxicity by CCl4 is due to enzymatic activation to released CCl3 radical in free state, which in turn disrupts the structure and function of lipid and protein macromolecule in the membrane of the cell. The stabilization of serum bilirubin, SGPT, SGOT, ALP and LDH levels by herbal formulation is a clear indication of the improvement of the functional status of liver cells. The increased levels of SGPT, SGOT, ALP, LDH, TB, and DB are the conventional indicator of liver injury. In the present study, a significant reduction in the level of SGPT, SGOT, ALP, LDH, TB, and DB as shown in the

Table 2 was observed in the groups of animals treated with 200 and 400 mg/kg, b.w of herbal formulation. Silymarin significantly reduced these levels to normal the results reveal the hepatoprotective effect of herbal formulation compared with the CCl4 -treated group. This finding can further be corroborated with histopathological studies. The histopathological examination clearly reveals that the hepatocytes, central vein, are almost normal in herbal formulation 200 and 400 mg/kg. treated group in contrast to the toxicant group as shown in the **Figure.4**.

Group	Treatment	SGPT (I.U/L)	SGOT (I.U/L)	ALP (I.U/L)	LDH (I.U/L)	TB (% mg)	DB (% mg)
Group I	Normal control (no treatment)	42 ± 4.6	51.3 ± 9.15	210 ± 21	135.7 ± 10.14	0.133 ± 0.01	0.144 ± 0.04
Group II	Toxicant control paracetamol (3 gm/kg)	119 ± 5.9	130 ± 28	320 ± 25	293.4 ± 10.80	0.266 ± 0.01	0.365 ± 0.11
Group III	Silymarin (50 mg/kg) + paracetamol (3 gm/kg)	49 ± 5.2	54 ± 1	170 ± 13	151.2 ± 26.65	0.140 ± 0.01	0.137 ± 0.02*
Group IV	HPF (100 mg/kg) + paracetamol (3 gm/kg)	78 ± 3.0**	78 ± 18	230 ± 6.5	190.5 ± 6.24*	0.170 ± 0.01	0.162 ± 0.02
Group V	HPF (200 mg/kg) + paracetamol (3 gm/kg)	63 ± 7.65	60 ± 12	190 ± 10**	157.8 ± 37.6	0.157 ± 0.02	0.134 ± 0.03*

Table.2. Effect of polyherbal formulation and silymarin on serum parameters in CCl4 -induced hepatic damage in rats

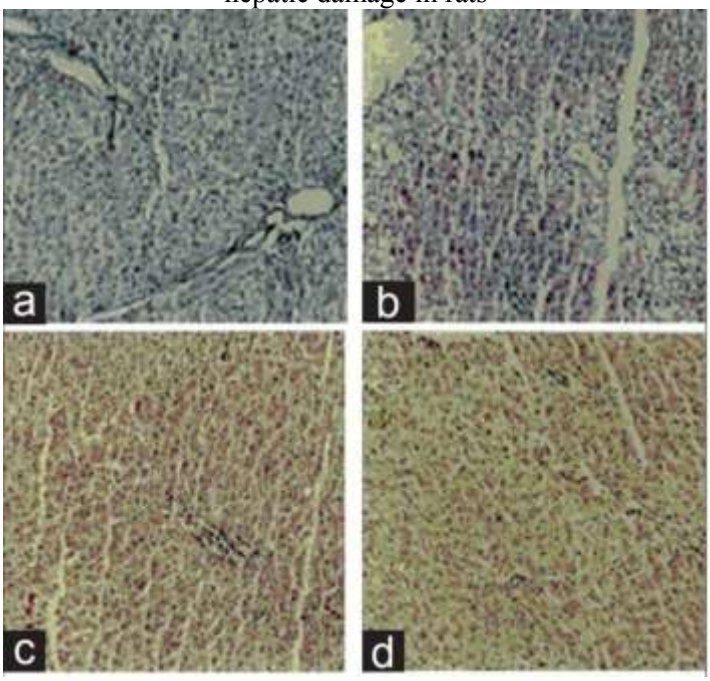


Figure.4. Histopathology of liver tissues. Histopathological study of carbon tetrachloride induced toxicity

3.5Alcohol-induced hepatotoxicity

Oxidative stress is one of the major factors in the etiology of ethanol injury mainly by Kupffer cell derived from ROS. Chronic consumption of ethanol causes injury to the liver cells. Increased level of serum albumin, AST, ALT, and ALP in alcohol-treated rats can be attributed to the damaged structural integrity of the hepatic cells because of the enzymes ALP located in the cytoplasm and released in the circulation after cellular damage. Alcohol consumption causes both plasma and organelle membrane damage. Alcohol consumption is known to cause fatty infiltration and cirrhosis. It enhanced lipid peroxidation produced during the microsomal metabolism of ethanol. It will directly generate free radicals such OH (hydroxy free radicals), and CH3 CHOH (hydroxy ethyl free radicals) through activation of cytochrome P-450 2E1 enzymes. Oral administration of ethanol at a dose of 30 ml/kg/day significantly increased the SGOT, SGPT, ALP, LDH, TB, and DB. Treatment with herbal formulation at the dose of 200 and 400 mg/kg/day along with alcohol showed significantly reduced levels of SGOT, SGPT, ALP, LDH, TB, and DB as shown in Table 3 as compared with control (alcohol treated). This shows that herbal formulation containing Salvia*miltiorrhiza* and *Tinosporacordifolia* to an extent preserves the structural integrity of the liver from the adverse effects of ethanol. Silymarin significantly reduced these levels to normal. The histopathological studies were performed to find out fatty changes, normal hepatic architecture, hepatocellular necrosis, and lymphocytic infiltration as shown in the **Figure 4**. Normal group showed no change, whereas rats treated with ethanol showed moderate to marked fatty changes micro vesicular to vacuolar. Herbal formulation treated group produced a marked degree of protection against ethanolinduced alterations, like those from normal rats.

Group	Treatment	SGPT (I.U/L)	SGOT (I.U/L)	ALP (I.U/L)	LDH (I.U/L)	TB (% mg)	DB (% mg)
Group I	Normal control (no treatment)	33 ± 4.6	51.3 ± 9.15	199 ± 21	135.7 ± 10.14	0.133 ± 0.01	0.144 ± 0.04
Group II	Toxicant control paracetamol (3 gm/kg)	110 ± 5.9	129 ± 28	320 ± 25	293.4 ± 10.80	0.266 ± 0.01	0.365 ± 0.11
Group III	Silymarin (50 mg/kg) + paracetamol (3 gm/kg)	49 ± 5.2	53 ± 1	170 ± 13	151.2 ± 26.65	0.140 ± 0.01	0.137 ± 0.02
Group IV	HPF (100 mg/kg) + paracetamol (3 gm/kg)	74 ± 3.0	76 ± 18	230 ± 6.5	190.5 ± 6.24	0.170 ± 0.01	0.162 ± 0.02
Group V	HPF (200 mg/kg) + paracetamol (3 gm/kg)	63 ± 7.65	59 ± 12	190 ± 10	157.8 ± 37.6	0.157 ± 0.02	0.134 ± 0.03

Table.3. Effect of polyherbal formulation and silymarin on serum parameters in ethanol-induced hepatic damage in rats

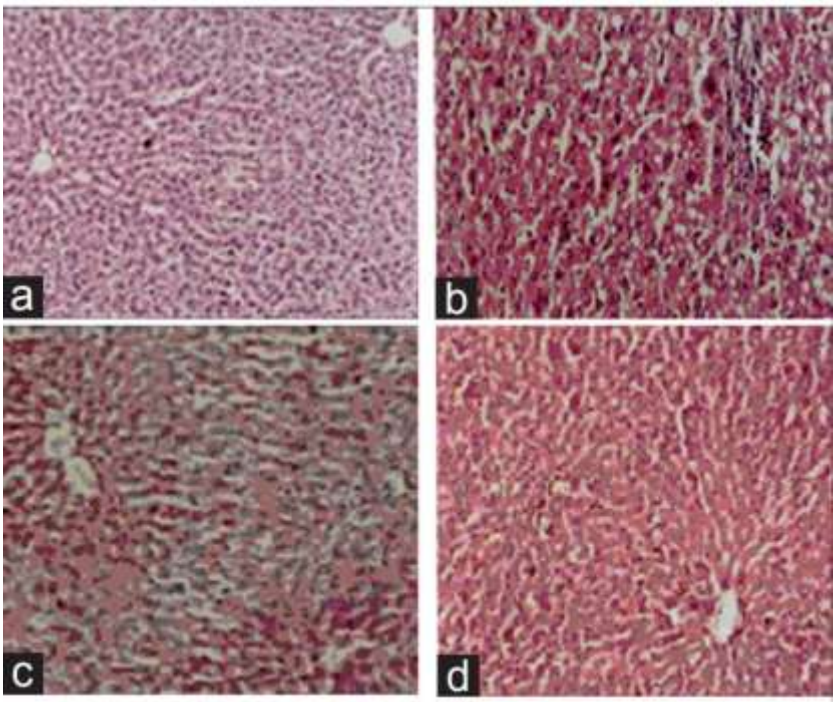


Figure.4. Histopathology of liver tissues. Histopathological study of ethanol-induced hepatotoxicity

4. Conclusion

Our finding confirmed the hepatoprotective effect of the herbal formulation and revealed that the herbal formulation has preventive action on paracetamol-, CCl4 -, and ethanolinduced hepatotoxicity in a dose-dependent manner. Herbal formulation significantly reduced the levels of SGPT, SGOT, ALP, LDH, TB, and DB. These findings are also confirmed by histopathological observation. Traditionally these plants are reported to have hepatoprotective activity. The resulting hepatoprotective activity of all three plants could be attributed to phytochemicals.

5. References

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