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EVALUATION OF HEPATOPROTECTIVE POLYHERBAL FORMULATION ON EXPERIMENTALLY INDUCED LIVER FIBROSIS IN WISTAR RATS

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ABSTRACT

Liver fibrosis is a significant worldwide well-being matter which results towards numerous chronic liver injuries, including hepatic viral illnesses, biliary diseases, and chemically-made liver disorders. Extracellular matrix (ECM) overproduction & its substitution of liver parenchymal tissue along fibrotic tissue are two characteristics of liver fibrosis. The existing treatment is only can stop further damage & there is no approve therapy for same. The study aim is to give the polyherbal formulation that will act on different pathophysiological steps of liver fibrosis. Six group of albino wistar rats were being experimented in this case, group I to VI i.e. normal control, disease control, standard control & test group of three different doses of polyherbal formulation (*Taraxacum Officinale* root extract, Fermented *Glycine max, Solanum nigrum* leaves extract, *Phyllanthus niruri* extract) at high dose, intermediate dose & low dose based on acute oral toxicity. Conducting the study is seen that polyherbal formulation of high dose in compare to that of standard group (silymarin) is showing better efficacy by detection of various biochemical parameters also by detecting the histopathological parameters of every group according to plan of work and protocol for evaluation of formulation. As further it will come in future work can be done into cell line on molecular base to determine clinical significance.

Keywords: Liver fibrosis, Tetracycline, polyherbal formulation, biochemical parameters, Silymarin

INTRODUCTION

Fibrosis is a typical liver reaction to a long-lasting abrasion brought through a diversity of aggressors, including congenital defects, viral infections, alcohol and medicaments misuse, self-immune attacks aiming liver cells & bile ducts, and metabolic disorders **[1-2]**.

In the Disse region belonging to regular liver, a structured collection about proteins identified as the extracellular matrix (ECM) may be situated close connection through the basement membrane. It supports the parenchymal cells and makes up roughly 0.5% of the liver's overall weight. Due to the ECM's non-fibrillar structure and ability to sustain the organ's architecture, chemicals can be exchanged between hepatocytes in a semi-continuous flow. This is essential for maintaining all liver cells' specialized tasks **[3]**.

The ECM components (collagen and others) in the fibrotic liver are alike to the particular cells exist in the healthy liver, by the exemption a particular the fibrosis has led to a quantitative augmentation of these components **[3]**.

The Kupffer cells' paracrine initiation of the HSC causes an upregulation & rearrangement of the comparative amounts of proteins of ECM, changing the sub-endothelial space matrix's normal structure towards in-between matrix along a huge quantity of thread like collagen. These amounts are first deposited in the central vein and/or portal tract, resulting in the creation of fibrous networks amongst the vessels like structures which results into eventual damage around capillarized and microvilli-rich sinusoidal endothelium and hepatocytes. In addition to having an impact on ECM growth, this also prevents the hepatic lobe from undergoing its typical vascularization, which impedes the organ's ability to operate.

These modifications underline the prime part related to ECM exist in liver, which involves acting for example a continuous network connecting the cells and facilitating continuous signal exchange via its own receptors in addition to serving as a framework for the organ's architecture. Fibrosis is an important biological event in and of itself because it results from an unevenness amongst the production in addition breakdown of the parts of extracellular matrix. Once allied through additional hepatic conditions, it encourages the enduring progress to cirrhosis, whatever in the nonappearance related to quick and appropriate dealing typically results in fatality **[4]**.

Millions of people around the world were impacted by chronic liver illnesses; out of 100% of population 30% of people suffering are prone to progress fibrosis and cirrhosis. In both sexes, the likelihood of having

substantial liver fibrosis increased with age. The new study found a link between older age and liver fibrosis, which is in line with earlier studies' findings. It has been suggested that increasing liver stiffness (LS) in the elderly population is pathophysiologic. With a fall in hepatic flow of blood related with age, which may upsurge the liver stiffnes, each hepatocyte volume and number is reduced. Matrix metalloproteinases' decreased collagenolytic activity may also cause liver fibrosis and collagen buildup in aging livers. Moreover, aging has an impact on the cellular and molecular systems that control liver regeneration.

The risk factors for Type-2 Diabetes Mellitus (T2DM), metabolic syndrome, and obesity were all linked to liver fibrosis; these conditions are all indicators of Non-alcoholic fatty liver disease (NAFLD). Due toward fact that T2DM as well NAFLD both share this underlying pathogenic mechanism of insulin resistance, NAFLD is a recurrent discovery which is present in diabetic individuals. Individuals suffering from T2DM who are sensitive individuals in addition to the Hepatitis B surface antigen possessing group of individuals had a higher occurrence of high-risk fibrosis of liver. Particularly in the progressive stage, fibrosis of liver remained strongly predicted by T2DM **[5]**.

In India, hepatic disorder is a complicated community health problem. Recent statistics from the World Health Organization show that 259,749 people have died in India from liver disease or 2.95% of all deaths. Age-adjusted mortality rates place India at 63rd in the world, at 22.93 deaths per 100,000 people **[6]**.

Around 100,000 people are affected by hepatic fibrosis worldwide, making it the world's top cause of death. 14.4% of people in the population have substantial liver fibrosis. Men and women who developed liver fibrosis began to age fast in their 40s and 50s, respectively. Men were more likely than women to have substantial liver fibrosis in each age group **[7-8]**.

According to Méndez-Sánches, 2 million new instances of chronic liver disorders (CLD) will be identified globally by the year 2050 **[9]**.

Liver fibrosis may be occurred by a diversity of antecedent aspects. Fibrosis in the liver can be initiated and advanced by genetic modifications, metabolic problems, cholestasis, parasites, viruses, toxins, ALD, which is a liver disease brought on by alcohol, as well as a host of other risk factors. NASH (non-alcoholic steatohepatitis), PNPLA3 (patatin-like phospholipase domain-containing protein 3), RNF7 (ring finger protein 7), and TGF- (transforming growth factor) are all abbreviations for the same condition. Autoimmune hepatitis is referred to as AIH.

C-X-C motif chemokine receptor 3 is known as CXCR3. Viral hepatitis B and C **[10]**.

Liver fibrosis is induced in rat by using Tetracycline antibiotics. The Tetracycline is given by oral route with using oral gavage in the amount of 140 milligram per kilogram of body weight per individual day along the duration of a week after 4 hours of administration of either polyherbal formulation (PHF) or standard drug. It works by causing an accumulation of lipids in the liver, which is further accompanied by LP, which increase in amount of ROS. This results into triggers chemokines which ultimately results into HSC production & causes trans differentiation of cells which results into ECM synthesis & causes fibrosis **[11]**.

Liver fibrosis is treated by using polyherbal formulation & it consists of following herbs: *Taraxacum officinale* root extract which acts by prevents collagen deposition, anti-inflammatory & anti-oxidant **[12]**. Fermented *Glycine max* acts as anti-inflammatory & by degrading collagens **[13]**. *Phyllanthus niruri* extract acts by anti-oxidant, by inhibiting lipid peroxidation, improves lipid profile as well as decreasing collagen content **[14]**. *Solanum nigrum* leaves extract acts by inhibiting HSC activation & by improving lipid profile **[15]**. The motive of project is to do the evaluation of low, intermediate &high dose of polyherbal formulation (dose calculated based on Acute oral toxicity study) against standard silymarin leaves extract tablet in experimentally caused liver fibrosis in albino wistar rats. Fibrosis of liver is introduced by Tetracycline by oral route in rats. The duration of induction is of 7 days. The treatment is of duration of 28 days.

MATERIAL AND METHOD

Herbs & excipients

Taraxacum officinale (Dandelion) root extract was procured from HealthyHey Nutrition, Mumbai, India. *Glycine max* (Soyabean) powder was procured from Loba Chemie Private Limited, Mumbai, India. *Phyllanthus niruri* (Bhui amla) extract was procured from Amsar Private Limited, Indore, India. *Solanum nigrum* (Black nightshade) leaves extract was procured from Neoteric DCBA Ideas, Coimbatore, India. Silymarin leaves extract water soluble tablet was procured from Alchem Phytoceuticals, Ballabgarh, India. Carboxymethyl cellulose (CMC) powder was procured from Central Drug House (P) Ltd., Dahej, India. Water for injection was procured from Otsuka Pharmaceutical India Private Ltd., Ahmedabad, India.

Drugs

Tetracycline water soluble powder was procured from SSS Vet Pharma, Ahmedabad, India.

Phytochemical Investigation

Phytochemical tests were carried out for extract of *Taraxacum officinale* root extract,

fermented *Glycine max*, *Phyllanthus niruri* extract and *Solanum nigrum* leaves extract to check presence of various phytochemical constituents. Various tests such as Dragendroff test, Mayer's test, Hager's test, Wagner's test, Molisch's test, Fehling's test, Benedict's test, Shinoda test, Legal test, Keller-killiani test, Foam test, Iodine test, Tannic acid test, Tannin's test by 5% $Fecl_3$, Tannin's test by Lead acetate, Tannin's test by Acetic acid, Tannin's test by Dilute HNO_3, Salkowski test, Liebermann-Burchard test, Terpenoid test by $CHCl_3$, Volatile oil test by tip of filter paper, Volatile oil test by Characteristics aroma, Bromine test, Coumarin Glycoside test by 10% NaoH, $FeCL_3$ test, Ninhydrin test, Million's test, Biuret's test and Gelatin test **[16]**.

Preparation of polyherbal suspension

Polyherbal formulation was administered to albino wistar rats in the form of **suspension.** Suspension was administered by the **oral route** of administration. The formulae for preparing a suspension of extracts of Taraxacum officinale root extract, fermented Glycine max, Phyllanthus niruri leaves extract & Solanum nigrum leaves extract were taken in the ratio of 2:2:1:0.36. The suspension was prepared by using various bioactive extracts of selected plant materials trituration method in mortar and pestle by using the suitable suspending agent of carboxy methyl cellulose (CMC) in the concentration of 0.5% w/v with continuous triturating. Lastly, to get a uniform product, the final volume was increased by adding pure water through continuous trituration in suspension [17].

Evaluation Parameters of Suspension

Redispersibility

The suspension was allowed to settle in a measuring cylinder. The cylinder's mouth was closed, inverted through 180 degrees, and the quantity of inversions required to re-establish a homogeneous suspension was calculated.

Rheology

The time required for each suspension sample to flow through a 10 ml pipette was determined by the apparent viscosity by using the equation

Flow rate = Volume of Pipette (ml)/ Flow rates of (seconds)

Particles size analysis

The distribution of particle size in suspension is an important aspect of its stability. Particle size distribution was carried out by using optical microscopy in dilute suspensions.

pH

Using a Elico LI 610 pH metre, the suspension's pH was determined.

Sedimentation volume

As the suspension settles in a cylinder under suitable standard conditions, the sedimentation volume is defined as the ratio of the final sediment height to the total

suspension's initial height. It was assessed by observing that the volume of the sediment is expressed as ultimate height and keeping a measured volume of suspension in a graduated cylinder in an undisturbed state for a certain amount of time.

Viscosity

Viscosity was measure using Labmaan LMDV 60 Brookfield's Viscometer **[17]**.

Animals

Healthy Albino wistar rats (Female) weighing 250-300g were used for the study. All of the studies and procedures reported in this study were received approval by the Institutional Animal Ethics Committee (IAEC) of Pharmacology department, Parul institute of pharmacy and research and with permission from Committee for the Control and Supervision of Experiments on Animals (CCSEA). Protocol No. PIPR 984/2022/02/02.Permitted Animals: 42 Animals were procured from Zydus Cadilla Research Center, Ahmedabad.

Housing

Albino Wistar rats were allowed for acclimatization for seven days on pelleted standard rat food with water and housed in a group of 3 rats per cage under well-controlled standard conditions of temperature (22±3ºC), humidity (30%-70%) and 12hrs light conditions and 12hrs dark condition cycle in animal house. Animals were given fed regularly with R.O. drinking water through polypropylene water bottles with SS spout ad libitum.

Acute oral toxicity study

OECD-423 (Acute Oral Toxicity-Acute Toxic Class Method) was performed on albino wistar rats to evaluate the oral toxicity dose of PHF as well for determining the highest, intermediate & lowest doses of PHF for treatment. As per the guideline, three female rats were administered a single starting dose of 2000mg/kg, and were observed for signs of toxicity and mortality. Animals were individually monitored after dosing at least once in the first 30 minutes, at regular intervals for the first 24 hours, with extra care taken in the first four hours, and daily thereafter, for a total of 14 days i.e., 2 weeks. After 14 days of daily observation animals were found to be normal. Animals were humanely sacrificed after the study was finished. Blood parameters were carried out & histopathology of the liver & kidney was done.

After the initial acute oral toxicity study confirmatory study was also carried out in the same manner as of initial study **[18]**.

Animal Groupings

Protocol for evaluation of polyherbal formulation for treatment of liver fibrosis. Animals were divided into 6 groups of six animals each.

Normal Control Group (NC)

Group-1: Received vehicle for 28 days (p.o.) & were given access to food &

drinking water.

Disease Control Group (DC)

Group-2: Each animal was given Tetracycline (140mg/kg bodyweight, p.o.) for 7 days.

Standard Control Group (SC)

Group-3: Each animal was given Tetracycline (140mg/kg bodyweight, p.o.) for 7 days along with Silymarin leaves extract (200mg/kg bodyweight, p.o.) for 28 days.

Test Group Polyherbal Low Dose (PHF-L)

Group-4: Each animal was given Tetracycline (140mg/kg bodyweight, p.o.) for 7 days along with PHF (100mg/kg bodyweight, p.o.) for 28 days.

Test Group Polyherbal Intermediate Dose (PHF-I)

Group-5: Each animal was given Tetracycline (140mg/kg bodyweight, p.o.) for 7 days along with PHF was given PHF (200mg/kg bodyweight, p.o.) for 7 days.

Test Group Polyherbal High Dose (PHF-H)

Group-6: Each animal was given Tetracycline (140mg/kg bodyweight, p.o.) for 7 days along with PHF (400mg/kg bodyweight, p.o.) for 28 days.

Induction procedure

Female albino rats weighing between 120-160g was selected. Animals were assimilated to laboratory condition for the period of 14 days. Tetracycline was administered by means of gastric tube. It was administered in the dose of 140 mg/kg body weight/day for the period of 7 days before feeding. At the end of dosing of this induction agent various liver parameters were evaluated as well as histopathological evaluation of liver was also to carried at the end of experiment by sacrificing the animals **[11, 19]**.

Evaluation of polyherbal formulation

Polyherbal formulation (PHF) efficacy was evaluated on the albino wistar rats. PHF was administered to three different groups of Rats. PHF was given at low dose, intermediate dose as well as at highest dose in three different group for the period of 28 days. Similarly standard drug i.e., Silymarin leaves extract was also administered at dose of 200mg/kg for the period of 28 days in SC group. Along with this one normal control group & disease control group was administered 0.5%w/v of CMC & Tetracycline respectively by oral route for the period of 28 days.

Blood collection

Blood was collected via retro-orbital route. The amount of blood collected was up to 1.5 ml. The blood was collected in blood clot activator vacuum tubes and eppendorf tubes and was evaluated by using different parameter evaluation kit by using Biochemical analyzer. The blood was at the end of the treatment i.e., on 28th day.

Biochemical estimation

Various biochemical parameters were carried out using different standard biochemical kits

Liver weight

After sacrificing animal liver was dissected and wash in normal saline and weigh on the weighing balance. Weight of liver was compared with each group. Weight variation was recorded.

Histopathological evaluation

Rats sacrificed at the end of the treatment and liver was remained, blotting free of blood and tissue fluids and preserved in 5 % formalin. After 24 hours the tissues were washed thoroughly with 70% alcohol and then dehydrated in ascending grades of alcohol (70-100%). Dehydration in absolute alcohol was followed by treatment of tissues with toluene: xylene (50:50) followed by paraffin wax in toluene and finally in 100% wax (paraffin wax, 60- 62°C) followed by embedding of tissue in wax. 5-15µm thick section were serially cut on a leitz microtome in horizontal plane and mounted on glass slide with the help of egg albumin in glycerine solution (50% v/v). They were then stained with 10% haematoxylin for 3-5 minutes and the staining was intensified by placing in running water. The haematoxylin-stained sections were stained with 10% eosin for 2 minutes and were then quickly passed through ascending grades of alcohol and finally treated with xylene followed by mounting in DPX. The sections were observed and required areas were photographed by Magnus Olympus cx23 photomicroscope. The sections were viewed under 40X and 100X magnifications.

Statistical analysis

All the values are expressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Sidak's multiple comparisons test as appropriate using computer based fitting program (Prism, GraphPad 9.5.0). Differences were considered to be statistically significant when $p<0.05$.

RESULTS

Phytochemical investigation

Methanolic extract of *Phyllanthus niruri,* Water extract of *Taraxacum officinale* root and *Solanum nigrum* leaves, Powder of *Glycine max* were exposed to phytochemical assessment for checking the occurrence of numerous chemical constituents **(Table 1)**.

Evaluation parameters of suspension

Polyherbal suspension was subjected to evaluation for various parameters such as Redispersibility, Flow rate, Particle size, pH, Sedimentation volume, Viscosity **(Table 2)**.

Acute oral toxicity study

OECD-423 (Acute Oral Toxicity-Acute Toxic Class Method) was performed on albino wistar rats.

Initial Study

Acute toxicity testing was done at a dosage

of 2000 mg/kg. skin, fur, eyes, and mucous membranes did not show any changes. Additionally, the respiratory, autonomic, central, and somatomotor nerve systems as well as the behavioral pattern were all normal. Tremors, convulsions, salivation, diarrhoea, lethargy, and coma were not seen **(Figure 1)**.

Confirmatory Study

Acute toxicity i.e., Confirmatory study was done at dosage of 2000mg/kg. skin, fur, eyes, and mucous membranes did not show any changes. Additionally, the respiratory, autonomic, central, and somatomotor nerve systems as well as the behavioral pattern were all normal. Tremors, convulsions, salivation, diarrhoea, lethargy, and coma were not seen **(Figure 2)**.

Evaluation of polyherbal formulation

Biochemical parameters

A. Liver function test

1. ALT (Figure 3)

In **Figure 3** ALT level of disease control group was severely increase in compared to normal control group. While, in compare to disease control group standard control group showed mild decrease in ALT level, similarly test low group showed mild decrease in ALT level, test intermediate was severely decrease in ALT level, test high group was severely decrease in ALT level in compared to disease control group.

2. AST (Figure 4)

In **Figure 4** AST level of disease control group was severely increase in compared to normal control group. While, test intermediate group showed moderate decrease in AST level and test high group showed severely decrease in AST level in compared to disease control group.

$^{\#}$(P<0.05), $^{\#\#}$ (P<0.01), $^{\#\#\#}$(P<0.001) compared to disease control.

3. Total Bilirubin (Figure 5)

In **Figure 5** Total bilirubin (TB) level of disease control group was severely increase in compared to normal control group. While, in compare to disease control group standard control group showed mild decrease in TB level, similarly test low group showed mild decrease in TB level, test intermediate was moderately decrease in ALT level, test high group was severely decrease in TB level in compared to disease control group.

4. Direct Bilirubin

In **Figure 6** Direct bilirubin (DB) level of disease control group was severely increase in compared to normal control group. While, in compare to disease control group standard control group showed mild decrease in DB level, similarly Test low group showed mild decrease in DB level, test intermediate was moderately decrease in DB level, test high group was severely decrease in DB level in compared to disease control group.

5. Indirect Bilirubin

In **Figure 7** Indirect bilirubin (IB) level of disease control group was severely increase in compared to normal control group. While,

in compare to disease control group test low group showed mild decrease in IB level, test intermediate showed mild decrease in IB level, test high group was moderately decrease in IB level in compared to disease control group.

6. Gamma-GT

In **Figure 8** GGT level of disease control group was severely increase in compared to normal control group. While, in compare to disease control group standard control group showed mild decrease in GGT level, similarly test low group showed mild decrease in GGT level, test intermediate was moderately decrease in GGT level, test high group was severely decrease in GGT level in compared to disease control group.

B. Lipid profile

1. HDL

In **Figure 9** HDL level of Disease control group was severely decrease in compared to normal control group. While, in compare to disease control group test high group was moderately increase in HDL level in compared to disease control group.

2. LDL

In **Figure 10** LDL level of Disease control group was severely increase in compared to normal control group. While, in compare to disease control group test intermediate group showed mild decrease in LDL level, while test high group was moderately decrease in LDL level in compared to disease control group.

3. Total Cholesterol (TC)

In **Figure 11** TC level of disease control group was severely increase in compared to normal control group. While, in compare to disease control group test intermediate group showed mild decrease in TC level, while test high group was moderately decrease in TC level in compared to disease control group.

4. Triglycerides (TG)

In **Figure 12** TG level of disease control group was severely increase in compared to normal control group. While, in compare to disease control group test intermediate group showed mild decrease in TG level, while test high group was moderately decrease in TG level in compared to disease control group.

5. VLDL

In **Figure 13** VLDL level of disease control group was severely increase in compared to normal control group. While, in compare to disease control group test intermediate group showed mild decrease in VLDL level, while test high group was moderately decrease in VLDL level in compared to disease control group.

6. Liver weight

In **Figure 14** Body weight of disease control group was severely decrease in compared to normal control group. While, in compare to disease control group standard control group showed mild increase in bodyweight, similarly test low group showed mild

increase in bodyweight, test intermediate was severely increase in bodyweight, test high group was severely increase in bodyweight in compared to disease control group.

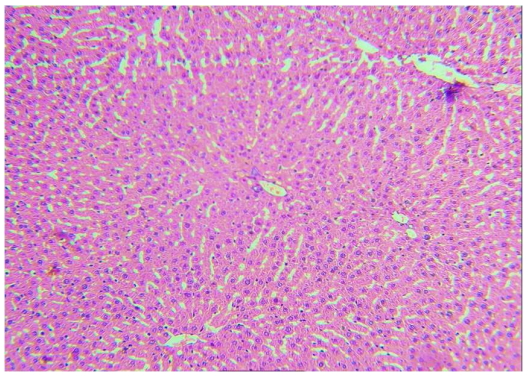
Table 1: Identification Test for Major Constituents

Types of Phytochemical Constituents	Methanolic extract of *Phyllanthus niruri*	Water extract of *Taraxacum officinale* root	Water extract of *Solanum nigrum* leaves	Powder of *Glycine max*
Alkaloid	+ve	+ve	+ve	+ve
Flavonoid	+ve	+ve	+ve	+ve
Carbohydrates	+ve	+ve	+ve	+ve
Cardiac Glycosides	+ve	+ve	+ve	+ve
Starch	+ve	+ve	+ve	+ve
Tannin	+ve	+ve	+ve	+ve
Saponin Glycosides	+ve	+ve	+ve	+ve
Terpenoid	+ve	+ve	-ve	+ve
Anthraquinone	-ve	-ve	-ve	-ve
Steroids	+ve	+ve	-ve	-ve
Volatile oil	+ve	-ve	+ve	-ve
Coumarin	+ve	+ve	+ve	-ve
Resin	+ve	+ve	+ve	-ve
Amino Acid	+ve	+ve	+ve	+ve
Protein	+ve	+ve	+ve	+ve
Phenol	+ve	+ve	+ve	+ve

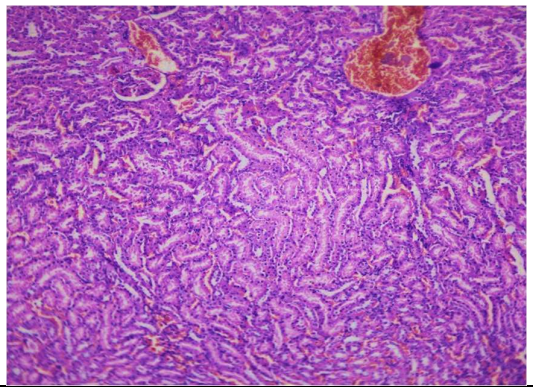
(+ve) Indicates positive result (-ve) Indicates negative result

Table 2: Evaluation Parameters of Suspension

Parameters	Result
Redispersibility	Good
Flow rate	0.9375 ml/sec
Particle size	20 µm
pH	5
Sedimentation volume	5 ml in 100 ml of suspension
Viscosity	158.9 mPa.s

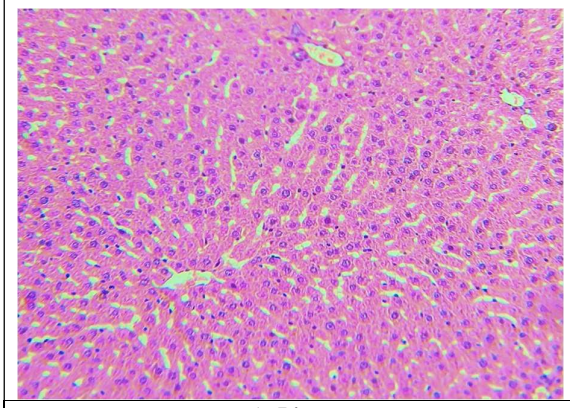


A. Liver
Normal histological structure of the central vein & demonstrating normal looking hepatic lobule & hepatocytes surrounding a central vein.



B. Kidney
Normal histological structure of the of glomeruli, tubules were seen.

Figure 1: Histological Investigation of liver & kidney (Initial Study)



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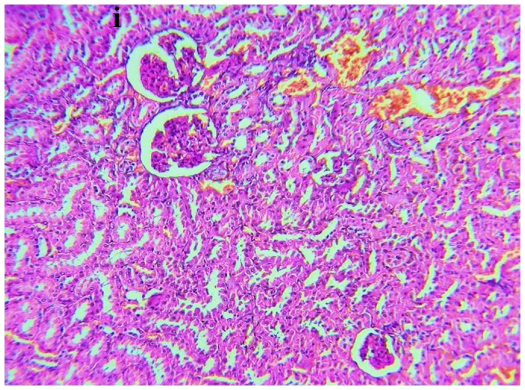

B. Kidney
Normal histological structure of the of glomeruli, tubules were seen.

Figure 2: Histological Investigation of liver & kidney (Confirmatory Study)

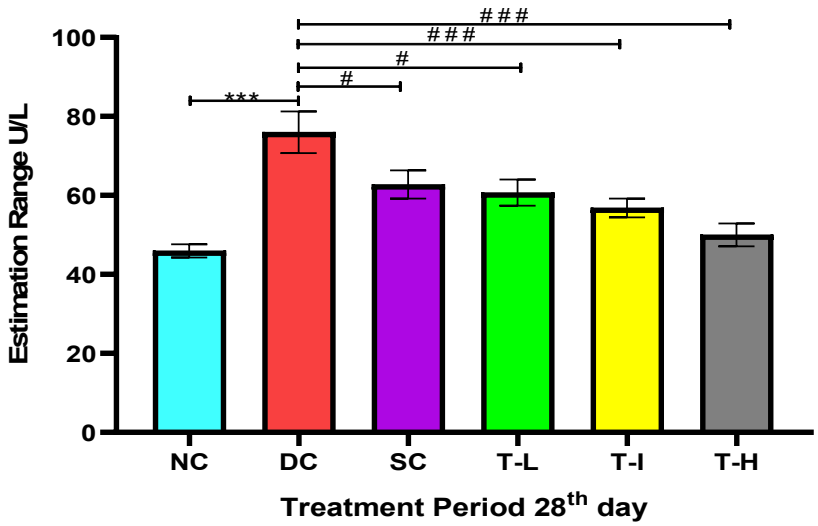

Figure 3: Effect of PHF on ALT levels in Tetracycline (140mg per kg per day p.o. for 1 week) in the liver fibrosis in wistar rats *(P<0.05), **(P<0.01), *(P<0.001) compared to normal control, while #(P<0.05), ##(P<0.01), ### (P<0.001) compared to disease Control**
All Values are expressed as Mean ± SEM (n=6) by ANOVA followed by Multiple comparison test.

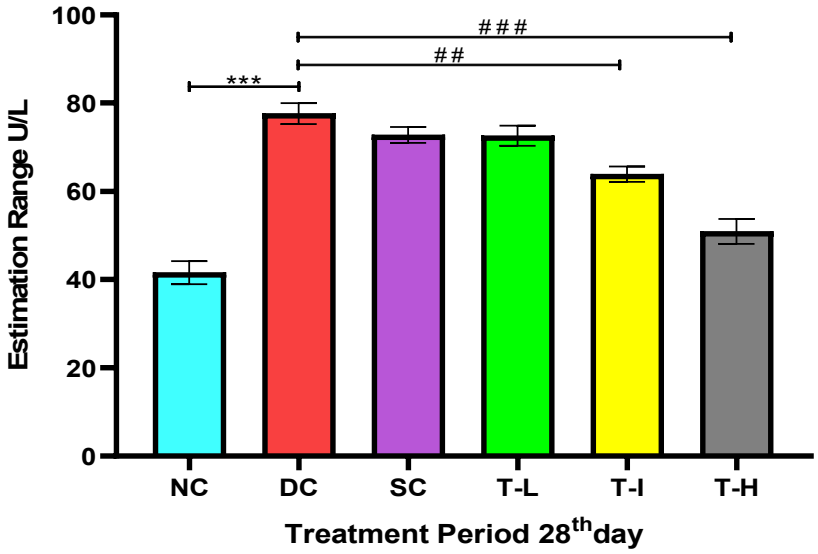

Figure 4: Effect of PHF on AST levels in Tetracycline (140mg per kg per day p.o. for 1 week) in the liver fibrosis in wistar rats *(P<0.05), **(P<0.01), *(P<0.001) compared to normal control, while #(P<0.05), ##(P<0.01), ### (P<0.001) compared to disease control**
All Values are expressed as Mean ± SEM (n=6) by ANOVA followed by Multiple comparison test.

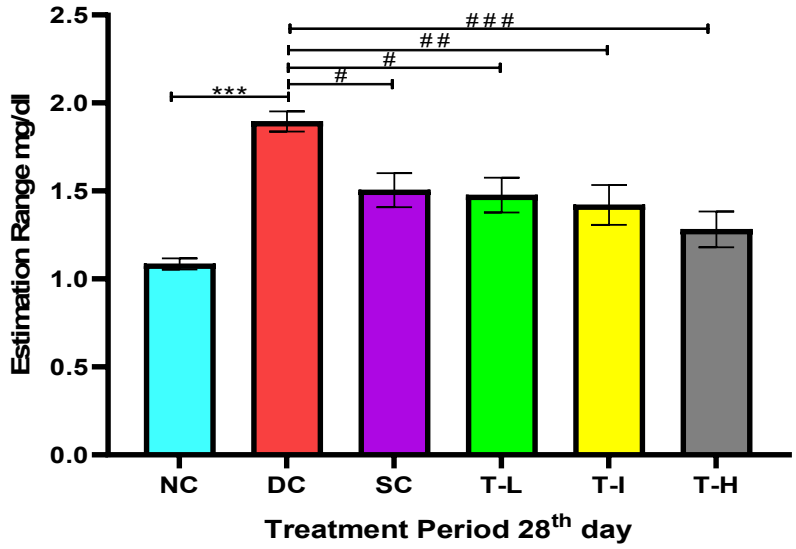

Figure 5: Effect of PHF on Total Bilirubin levels in Tetracycline (140mg per kg per day p.o. for 1 week) in the liver fibrosis in wistar rats *(P<0.05), **(P<0.01), *(P<0.001) compared to normal control, while #(P<0.05), ##(P<0.01), ### (P<0.001) compared to disease control.**
All Values are expressed as Mean ± SEM (n=6) by ANOVA followed by Multiple comparison test.

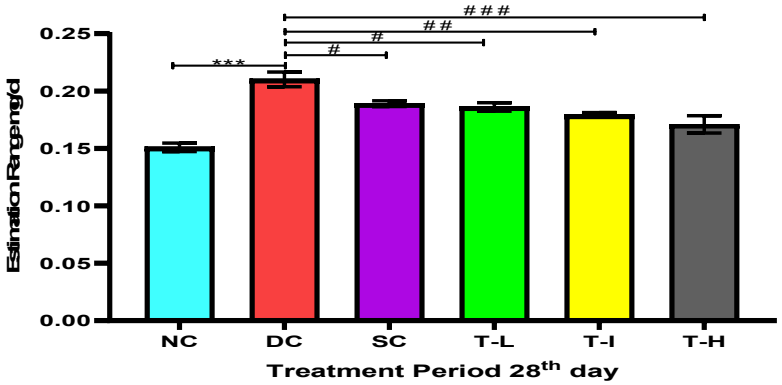

Figure 6: Effect of PHF on Direct Bilirubin levels in Tetracycline (140mg per kg per day p.o. for 1 week) in the liver fibrosis in wistar rats *(P<0.05), **(P<0.01), *(P<0.001) compared to normal control, while #(P<0.05), ##(P<0.01), ###(P<0.001) compared to disease control**

All Values are expressed as Mean ± SEM (n=6) by ANOVA followed by Multiple comparison test.

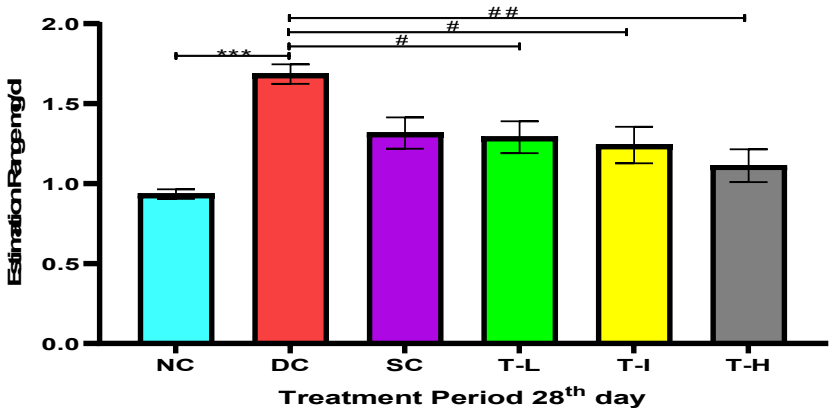

Figure 7: Effect of PHF on Indirect Bilirubin levels in Tetracycline (140mg per kg per day p.o. for 1 week) in the liver fibrosis in wistar rats *(P<0.05), **(P<0.01), *(P<0.001) compared to normal control, while #(P<0.05), ##(P<0.01), ###(P<0.001) compared to disease Control**

All Values are expressed as Mean ± SEM (n=6) by ANOVA followed by Multiple comparison test.

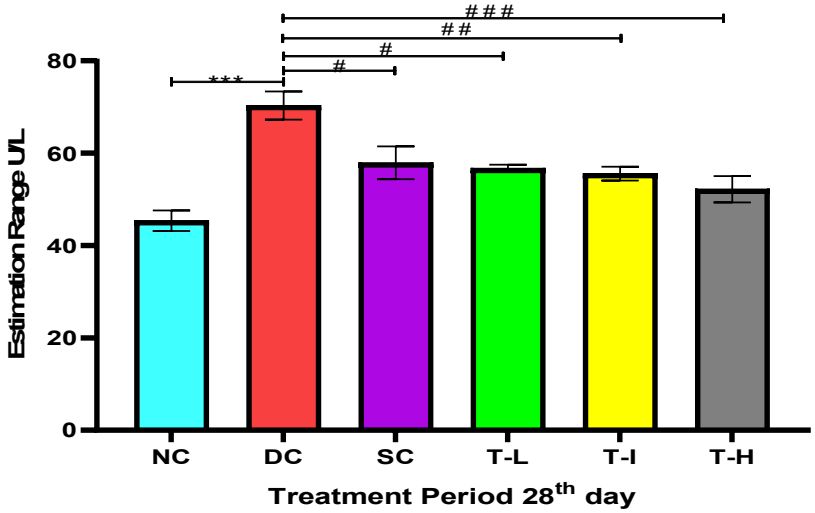

Figure 8: Effect of PHF on Gamma-GT levels in Tetracycline (140mg per kg per day p.o. for 1 week) in the liver fibrosis in wistar rats *(P<0.05), **(P<0.01), *(P<0.001) compared to normal control, while #(P<0.05), ##(P<0.01), ###(P<0.001) compared to disease Control**

All Values are expressed as Mean ± SEM (n=6) by ANOVA followed by Multiple comparison test.

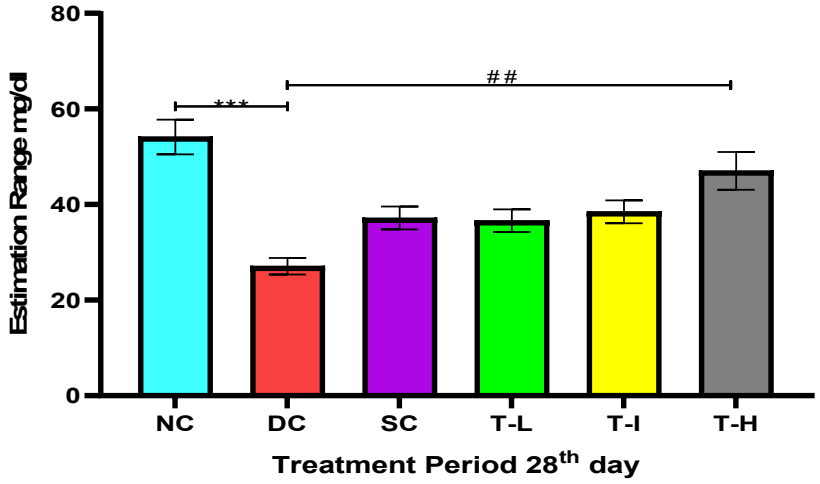

Figure 9: Effect of PHF on HDL levels in Tetracycline (140mg per kg per day p.o. for 1 week) in the liver fibrosis in wistar rats. *(P<0.05), **(P<0.01), *(P<0.001) compared to normal control, while #(P<0.05), ##(P<0.01), ## #(P<0.001) compared to disease control.**
All Values are expressed as Mean ± SEM (n=6) by ANOVA followed by Multiple comparison test.

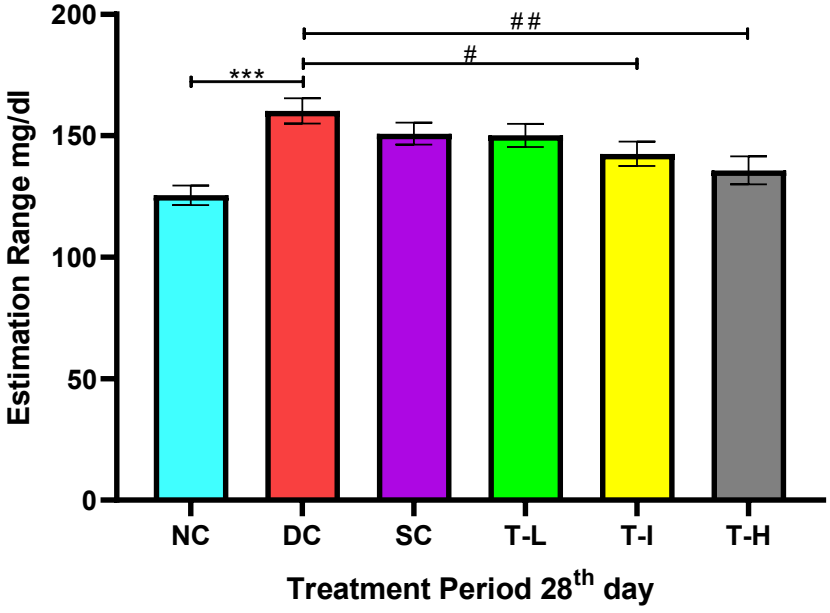

Figure 10: Effect of PHF on LDL levels in Tetracycline (140mg per kg per day p.o. for 1 week) in the liver fibrosis in wistar rats. *(P<0.05), **(P<0.01), *(P<0.001) compared to Normal control, while #(P<0.05), ##(P<0.01), ## #(P<0.001) compared to Disease Control**
All Values are expressed as Mean ± SEM (n=6) by ANOVA followed by Multiple comparison test

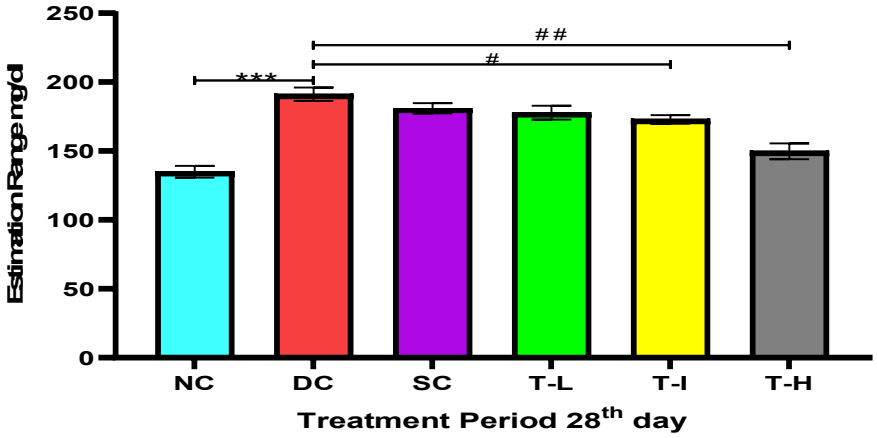

Figure 11: Effect of PHF on Total Cholesterol (TC) levels in Tetracycline (140 per kg per day p.o. for 1 week) in the liver fibrosis in wistar rats. *(P<0.05), **(P<0.01), *(P<0.001) compared to normal control, while #(P<0.05), ##(P<0.01), ###(P<0.001) compared to disease Control.**
All Values are expressed as Mean ± SEM (n=6) by ANOVA followed by Multiple comparison test.

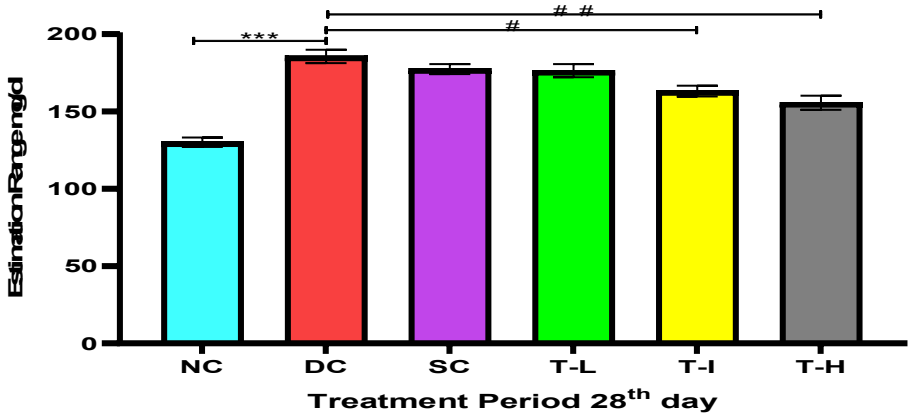

Figure-12 Effect of PHF on Triglycerides (TG) levels in Tetracycline (140mg per kg per day p.o. for 1 week) in the liver fibrosis in wistar rats. *(P<0.05), **(P<0.01), *(P<0.001) compared to Normal control, while #(P<0.05), ##(P<0.01), ###(P<0.001) compared to disease control.**
All Values are expressed as Mean ± SEM (n=6) by ANOVA followed by Multiple comparison test.

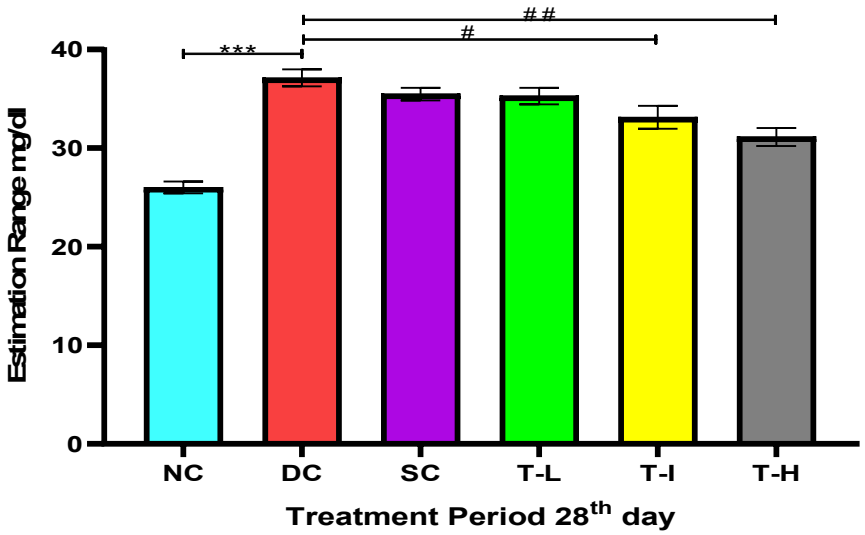

Figure 13: Effect of PHF on VLDL levels in Tetracycline (140mg per kg per day p.o. for 1 week) in the liver fibrosis in wistar rats. *(P<0.05), **(P<0.01), *(P<0.001) compared to normal control, while #(P<0.05), ##(P<0.01), ### (P<0.001) compared to disease Control.**
All Values are expressed as Mean ± SEM (n=6) by ANOVA followed by Multiple comparison test.

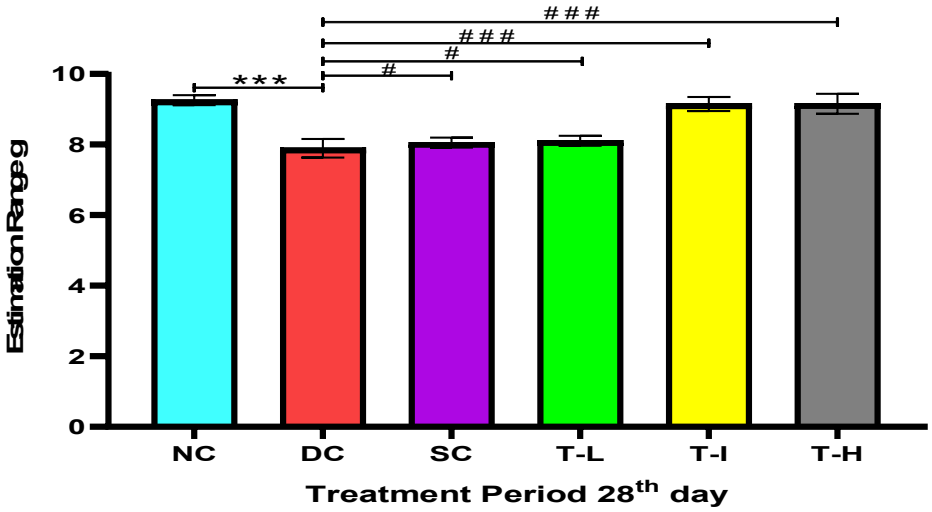

Figure 14: Effect of PHF on Liver Weight in Tetracycline (140mg per kg per day p.o. for 1 week) in the liver fibrosis in wistar rats. *(P<0.05), **(P<0.01), *(P<0.001) compared to normal control, while #(P<0.05), ##(P<0.01), ### (P<0.001) compared to disease Control**
All Values are expressed as Mean ± SEM (n=6) by ANOVA followed by Multiple comparison test.

Histopathological investigation

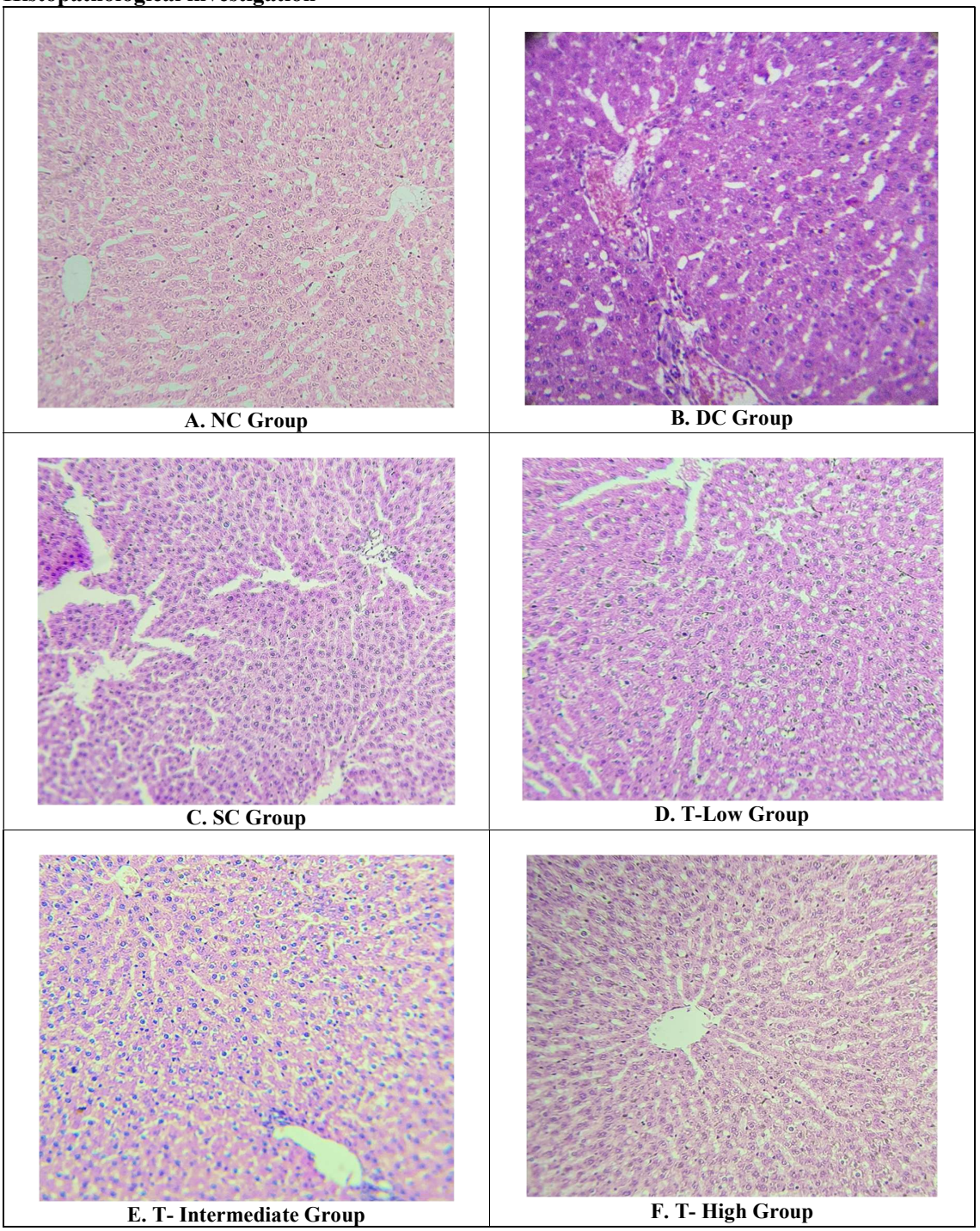

Figure 15: Histopathology

DISCUSSION

Liver fibrosis is a equilibrium among liver restoration and scar creation that results from either acute or chronic cellular injury. It is a revocable wound curative effect. Acute illnesses like hepatitis are linked to brief and transitory changes in the architecture of the liver. Unfortunately,

chronic diseases are considered by the continuous replacement of liver parenchymal cells by scar tissue. The persistent creation of scars is symbolized by continual hepatocyte regeneration. A loss of the physiological mechanisms of matrix turnover and an increase in the synthesis of matrix components characterize the dynamic process of liver fibrosis **[1, 20]**.

The animals of group (II – VI) were given orally Tetracycline water soluble powder at dose of 140mg/kg/day p.o. for 7 days dissolved in water for injection for the induction of liver fibrosis. Before initializing of treatment biochemical estimation was performed and was compared to normal control Group (Group-I). Normal control Group (Group-I) was considered as base line for biochemical analysis **[11, 19]**.

Tetracycline is an antibiotic which is bacteriostatic in nature having broad spectrum of antimicrobial activity. In prior research, Tetracycline significantly raised the levels of several biochemical markers in rats, including triglycerides, LDL, AST, ALT, bilirubin, urea, creatinine, and GGT. In addition to this, it significantly lowers the degree of albumin, total protein, and HDL. Tetracycline's effects on the liver included high levels of cytoplasmic vacuolation in hepatic cells, sinusoidal dilatation, and cell membrane loss in certain hepatocytes. Additionally, it has effects on the rat kidneys, including necrosis and vacuolation of the renal tubules, enlargement of the Bowman's gap, and shrinkage of the glomeruli **[11, 19]**.

In our study Tetracycline showed effects on biochemical parameters like rises in the level of ALT, AST, total bilirubin, direct bilirubin, indirect bilirubin, GGT, total cholesterol, triglycerides, LDL, VLDL. Also results into reduction in the degree of HDL as well causes reduction in the weight of liver. Histopathological changes like structure of the central vein was moderately damaged but less in compare to disease control and demonstrating abnormal damaged looking hepatic lobule as well hepatocytes surrounding a central vein. There was slight sinusoidal dilation as well as disorientation of cells were present, but no sign of necrosis. There was negligible deposition of collagen.

Tetracycline works by causing an accumulation of lipids in the liver, which is followed by lipid peroxidation, which causes more reactive oxygen species (ROS) generation. It results into increases in chemokines & cytokines levels which in turns activates quiescent hepatic stellate cells to undergoes transdifferentiation to forms myofibroblast, which in turns causes extracellular matrix as well collagen deposition & finally results into liver fibrosis **[11]**.

Polyherbal formulation (PHF) was consists

of *Taraxacum officinales* root extract, fermented *Glycine max*, *Phyllanthus niruri* extract and *Solanum nigrum* leaves extract. These all were combined on the basis of effects of these herbs on different pathophysiological steps of liver fibrosis.

Taraxacum officinale root extract possess hepatic stellate cells (HSC) inactivation and collagen deposition inhibition activity by decreases the expression of type- 1 and type-3 collagen. Also possess anti-inflammatory activity by decreases the myeloperoxidase activity and inhibit inflammatory markers and possess anti-oxidant activity by scavenging ROS and decrease the level of MDA **[12, 21]**.

Fermented *Glycine max* possess anti-inflammatory action by inhibiting inflammatory nitric oxide synthase and thus depresses transcription factor nuclear factor kappa-B and thus deceases inflammation. Also possess liver tissue repair activity because of presence of enzyme Serine protease enzyme which causes degradation of collagen and fibrin thus it helps hepatocyte to proliferate to fulfil necrotic area **[13, 21]**.

Phyllanthus niruri extract possess anti-oxidant activity by increasing level of glutathione thus it results into the level of SOD also causes decrease in lipid peroxidation and decreases the oxidative stress. Also possess lipid profile normalizing action by decreasing the Hydroxy methyl glutaryl-coenzyme A reductase enzyme (HMG-CoA) and thus normalize lipid profile. Also possess decrease in total collagen content by down regulating TGF-β1 activity which results into decrease in expression of Smad-2,3 **[14, 21]**.

Solanum nigrum leaves extract possess HSC inactivation activity by inhibiting MMP2 and α-SMA expression so thus decreases the transdifferentiation of activated HSC into myofibroblast. Also possess lipid profile normalizing action by suppressing fatty acid synthase and by also increase in PPAR-γ activity **[15, 21]**.

After 28 days i.e., at the conclusion of study it is evident that Group-II (disease control group) has significant abnormal increase in Alanine transaminase (ALT), Aspartate transaminase (AST), Gamma glutamyl transferase (GGT), Total bilirubin (TB), Direct bilirubin (DB), Indirect bilirubin (IB), Total cholesterol (TC), Low density lipoprotein (LDL), Very low-density lipoprotein (VLDL), Triglyceride (TG) levels and abnormal decreases in High density lipoprotein (HDL) and Liver weight. Also, abnormal histopathological interpretation of Histological structure of the central vein & demonstrating normal looking hepatic lobule as well hepatocytes surrounding a central vein. There was no sinusoidal dilation, no sign of necrosis as well as orientation of cells also were normal

and showed no signs of collagen deposition. On the contrary, in Group-VI (test group polyherbal high dose) shows significant decrease in ALT, AST, GGT, TB, DB, IB levels also TC, LDL, VLDL, TG levels and increases in HDL as well liver body weight. Also, histopathological interpretation almost resembles normal control group.

While in Group-V (test group polyherbal intermediate dose) shows moderate decrease in ALT, AST, GGT, TB, DB, IB levels also TC, LDL, VLDL, TG levels and slight increases in HDL as well liver body weight. Histological structure of the central vein was very slightly damaged in certain portion but less in compare to test low group and demonstrating almost normal looking hepatic lobule as well hepatocytes surrounding a central vein. There were negligible signs of sinusoidal dilation as well as orientation of cells were also almost normal, also no sign of necrosis. There was no deposition of collagen.

Group-III (test group polyherbal low Dose) shows mild decrease in ALT, AST, GGT, TB, DB, IB levels also TC, LDL, VLDL, TG levels and mild increases in HDL as well liver body weight.

Similarly, Group-II (standard control group) i.e., silymarin group having almost equivalent effects like that of Group-III on this all parameters and also in histopathological changes of liver. Histological structure of the central vein was very slightly damaged in certain portion but less in compare to test low group and demonstrating almost normal looking hepatic lobule as well hepatocytes surrounding a central vein. There were negligible signs of sinusoidal dilation as well as orientation of cells were also almost normal, also no sign of necrosis. There was no deposition of collagen.

While Group-I (normal control group) possess normal level of all these biochemical parameters and also normal histopathological interpretation.

Results of research showed that this polyherbal formulation (PHF) works in liver of induced Tetracycline rats by antioxidant, anti-inflammatory, prevents collagen deposition, HSC inactivation, repairs liver tissue as well by lowering cholesterol. So PHF by above mechanisms it scavenges ROS, inhibits cytokines & chemokines thus provides anti-inflammatory effect, provides HSC as well as collagen inhibitory effect and also repairs liver tissue by breakdown of collagen and aids to recover liver tissues.

Thus, this polyherbal formulation has promising effect to protect as well to cure Liver fibrosis.

CONCLUSION

Liver fibrosis is a one of the major hepatological disorder that affect liver causing scaring i.e., replacement of normal hepatocytes with scaring tissue and

collagen deposition on liver which in turns affect the various major functions of liver. As liver is responsible of various important functions of the body so it ultimately, affects entire body and if timely treatment is not providing it may turn fatal. Liver fibrosis develops and progresses as a result of numerous factors. The modern medication is effective up to certain extent and it possess side-effects also. Liver fibrosis is induced by Tetracycline antibiotic at dosage of 140mg per kg per day per oral for duration of 1 week. Induction causes rise in the level of liver function test and also results into abnormal lipid profile and causes abnormal histopathological changes in liver and collagen deposition. Thus, polyherbal formulation (PHF) normalize the level of liver function test as well lipid profile level and also normalize histopathology of liver by providing antioxidant, anti-inflammatory, prevents collagen deposition, HSC inactivation, prevents collagen synthesis, repair liver tissue, cholesterol lowering. All of the observations and measurements conducted in this study give preliminary proof that the PHF extract can be used to stop the advancement of liver fibrosis in rats that has been caused by Tetracycline. In particular, this natural extract has the ability to safeguard the liver by halting the adverse reactions brought on by Tetracycline poisoning. The effects are similar to those of silymarin, and the PHF extract's ability to maintain the liver's normal properties, structure, and function in the face of toxic exposure is encouraging. This calls for additional research examining the significance of the PHF extract's pharmacologic potential in treating liver fibrosis by identifying the molecular pathways of action as well as cell line studies.

Abbreviations

ECM-Extracellular matrix, LS-Liver stiffness, T2DM- Type-2 Diabetes Mellitus, NAFLD- Non alcoholic fatty liver disease, CLD-Chronic liver disorders-Alcoholic liver disease, AIH- Autoimmune Hepatitis, CXCR3- C-X-C motif chemokine receptor 3, HBV- Hepatitis B virus, HCV- Hepatitis C virus, IL28B-Interleukin 28B, NASH- Non-alcoholic steatohepatitis, PNPLA3- Patatin-like phospholipase domain-containing protein 3, RNF7- Forring finger protein 7, TGF-β-Transforming growth factor-β, PHF-Polyherbal formulation, ROS- Reactive oxygen species, ALT- Alanine transaminase, AST- Aspartate transaminase, TB- Total Bilirubin, DB-Direct Bilirubin, IB- Indirect Bilirubin, TC-Total Cholesterol, TG- Triglycerides, HDL- High density lipoprotein, LDL- Low density lipoprotein, VLDL- Very low-density lipoprotein, GGT- Gamma

glutamyl transferase, HSC- Hepatic stellate cells.

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Ethics approval- The protocol was conducted in accordance with guidelines of CCSEA and Protocol Number- PIPR 984/2022/02/02.

Conflict of Interest- There is no conflict of interest between Authors.

REFERENCES

[1] Parola M, Pinzani M. Liver fibrosis: Pathophysiology, pathogenetic targets and clinical issues. Molecular Aspects of Medicine. 2019; 65:37–55. doi: https://doi.org/10.1016/j.mam.2018.09.002.

[2] Rockey DC, Bell PD, Hill JA. Fibrosis - a common pathway to organ injury and failure. New England Journal of Medicine. 2015; 372(12):1138–49. doi: https://doi.org/10.1056/nejmra1300575.

[3] Povero D, Busletta C, Novo E, di Bonzo LV, Cannito S, Paternostro C, Parola M.Liver fibrosis: a dynamic and potentially reversible process. Histology and Histopathology. 2010;(8):1075-91.doi: https://doi.org/10.14670/hh-25.1075

[4] Sanchez-Valle V, C. Chavez-Tapia N, Uribe M, Mendez-Sanchez N. Role of oxidative stress and molecular changes in liver fibrosis: A Review. Current Medicinal Chemistry. 2012;19(28):4850–60.doi: https://doi.org/10.2174/092986712803341520.

[5] Nah E-H, Cho S, Kim S, et al. Prevalence of liver fibrosis and associated risk factors in the Korean general population: a retrospective cross-sectional study. BMJ Open 2021;11: e046529.doi: https://doi.org/10.1136/bmjopen-2020-046529.

[6] Choudhuri G, Chaudhari S, Pawar D, Roy DS. Etiological Patterns, Liver Fibrosis Stages and Prescribinga Patterns of Hepato-Protective Agents in Indian Patients with Chronic Liver Disease. Journal of The Association of Physicians of India. 2018; 66:1–8.doi: https://pubmed.ncbi.nlm.nih.gov/31315327/.

[7] Caballería L, Pera G, Arteaga I, Rodríguez L, Alumà A, Morillas R et al. High Prevalence of Liver Fibrosis Among European Adults with Unknown Liver Disease: A Population-Based Study. Clinical Gastroenterology and Hepatology. 2018;16(7): 1138-1145.doi; https://doi.org/10.1016/j.cgh.2017.12.048.

[8] Ramakrishnan A, Velmurugan G, Somasundaram A, Mohanraj S, Vasudevan D, Vijayaragavan P et al. Prevalence of abnormal liver tests and liver fibrosis among rural adults in low and middle-income country: A cross-sectional study. eClinicalMedicine. 2022;51: 101553.doi: https://doi.org/10.1016%2Fj.eclinm.2022.101553.

[9] Nunes Callado A, Maria Pinheiro Bezerra I, Adami F, Vinícius de Alcantara Sousa L, Carlos de Abreu L. Mortality and hospitalization for liver disease in the western Amazon from 2008 to 2017. Journal of Human Growth and Development. 2021;31(1):116–24. doi: http://dx.doi.org/10.36311/jhgd.v31.11066.

[10] Altamirano-Barrera A, Barranco-Fragoso B, Méndez-Sánchez N. Management Strategies for Liver Fibrosis. Annals of Hepatology. 2017;16(1): 48-56.doi: https://doi.org/10.5604/16652681.1226814

[11] Shabana M, Ibrahim H, Khadre S, Elemam M. Influence of rifampicin and tetracycline administration on some biochemical and histological parameters in albino rats. The Journal of Basic & Applied Zoology. 2012;65(5): 299-308.doi: http://dx.doi.org/10.1016/j.jobaz.2012.10.009.

[12] Hamza A, Mohamed M, Lashin F, Amin A. Dandelion prevents liver fibrosis, inflammatory response, and oxidative stress in rats. The Journal of Basic and Applied Zoology. 2020;81(1):43-56. doi: https://doi.org/10.1186/s41936-020-00177-9.

[13] Permata F, Roosdiana A, Anggraini V. The effect of fermented Glycine max (L.) Merr. to rats induced CCl4 toward hepatic IL-6 expression and ALT-AST level. Journal of Physics: Conference Series. 2020;1430(1):012019. doi: 10.1088/1742-6596/1430/1/012019.

[14] Al Zarzour R, Ahmad M, Asmawi M, Kaur G, Saeed M, Al-Mansoub M, et al. Phyllanthus niruri standardized extract alleviates the progression of non-alcoholic fatty liver disease and decreases atherosclerotic risk in Sprague–dawley rats. Nutrients.

2017;9(7):766. doi: https://doi.org/10.3390/nu9070766

[15] Tai C, Choong C, Shi Y, Lin Y, Wang C, Lee B et al. Solanum nigrum Protects against Hepatic Fibrosis via Suppression of Hyperglycemia in High-Fat/Ethanol Diet-Induced Rats. Molecules. 2016;21(3):269. doi: https://doi.org/10.3390/molecules21030269.

[16] Khandelwal KR, Practical Pharmacognosy, 28th Edition. Nirali Prakashan, Pune.2017: 25.1-25.6.

[17] Mahajan SM, Baviskar DT. Formulation development and evaluation of polyherbal suspension of some medicinal plants. International Journal of Current Research and Review. 2021;13(12):99–106.

[18] Test No. 423: Acute Oral toxicity - Acute Toxic Class Method. OECD Guidelines for the Testing of Chemicals, Section 4. 2002.

[19] Asha K, Sankar T, Nair P. Effect of tetracycline on pancreas and liver function of adult male albino rats. Journal of Pharmacy and Pharmacology. 2007;59(9):1241-1248. doi: https://doi.org/10.1211/jpp.59.9.0008

[20] Liedtke C, Nevzorova Y, Luedde T, Zimmermann H, Kroy D, Strnad P et al. Liver Fibrosis—From Mechanisms of Injury to Modulation of Disease. Frontiers in Medicine. 2022;8(1):1-27. doi: https://doi.org/10.3389%2Ffmed.2021.814496

[21] Janve YS, Kakadiya J, Raja M. An Updated Review on Medicinal Plants with Hepato-protective Activity. Journal of Natural Remedies. 2023Jan;23(1):47–55. doi: 10.18311/jnr/2023/30924.