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Hepatoprotective Evaluation Of Agniprabha Vati Against Paracetamol - Induced Liver Damage In Rats

Dr. Vaishnavi B R

PG Scholar, Department of Rasashastra and Bhaishajya Kalpana,
Ayurveda Mahavidyalaya Hubbali

Abstract:

Background: The liver plays a crucial role in metabolism, detoxification, and homeostasis. Drug-induced liver injury (DILI), especially by agents like paracetamol, remains a major cause of hepatic dysfunction globally.

Objective: To evaluate the hepatoprotective effect of Agniprabha Vati, a classical Ayurvedic herbo-mineral formulation, against paracetamol-induced hepatotoxicity in Wistar albino rats.

Methods: Albino rats were divided into five groups: Positive control, Negative control (paracetamol), Standard drug (silymarin), and Two test groups receiving Agniprabha Vati at therapeutic and double therapeutic doses. Hepatotoxicity was induced by oral administration of paracetamol (3g/kg). Test drugs were administered for 10 consecutive days. Liver function was assessed by serum biochemical markers (SGOT, SGPT, ALP, bilirubin, protein, and albumin), ponderal changes, and histopathology.

Results: Paracetamol administration caused significant hepatic damage, evidenced by elevated liver enzymes and structural changes in liver histology. Agniprabha Vati at both doses showed hepatoprotective effects, evident from normalization of biochemical markers and improvement in hepatic architecture. Results were comparable to silymarin, though statistically non-significant in some parameters.

Conclusion: Agniprabha Vati demonstrated hepatoprotective activity in paracetamol-induced liver injury in rats, supporting its classical indication in Yakrit (liver) disorders. Further clinical validation and long-term safety studies are warranted.

Keywords: Agniprabha Vati, Hepato-protection, Paracetamol-induced hepatotoxicity, Ayurvedic herbo-mineral formulation, Silymarin, Liver function tests, Histopathology

I. INTRODUCTION

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. Therefore, maintenance of a healthy liver is essential for the overall wellbeing of an individual.

Liver disease is a collective term for a whole group of problems that afflict the tissues, structures & cells of the human liver. Liver diseases are mainly caused due to excess use of alcohol, poor diet, malnutrition, infections & drug induced liver injury.

There are many plants & formulations available for the treatment of liver diseases mainly which possess hepatoprotective property.

Agniprabha Vati[1] specially prepared by using Rasasindhura, Saidhava lavana. Navasadara, Yavakshara, Vida lavana was also indicated in Yakrita & Pliha vikaras (liver & spleen disorders) by our Rasacharyas, so to assess the rationality behind the statement, the assessment of Hepatoprotective activity study of Agniprabha Vati was undertaken.

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The Includes;

- ✓ Materials and methods
- ✓ Observation & Results
- ✓ Histopathological Plates of liver tissues.
- ✓ Photos of Hepatoprotective Study

A. MATERIALS & METHODS

MATERIALS

The materials & techniques used in the present work are described in the following

Test drug- Agniprabha Vati

Standard Hepatoprotective drug- The reference standard drug used for hepatoprotective evaluation is silymarin. It was purchased from the market with the trade name Silybon-70. Mfg. Lic NO:M/600/2012, Mfd- July 2023, Exp-June 2026, Manufactured by micro labs limited, Mamring, Namthang Road, Namchi-737 132, Sikkim.

Toxicant- The toxicant used to induce hepatic injury is paracetamol-3g/k.g, given orally. Paracetamol–Dolo 650, Microlabs Limited, Mamring, Namthang Road, Namchi-737 132, Sikkim.

Mfg. Lic. No:M/600/2012, B.No: 00HS3741, Mfd- Aug-2024 , Exp-July-2028.

Chemicals- All the chemicals & reagents used in the experimental study were procured from standard & reputed firms and are of analytical grade regularly used in the laboratory.

Animals- Wistar strain albino rats of either sex of body weight ranging from 150 to 250 g. and which are in healthy condition were selected for present study. They were obtained from S. D. M. Centre for Research in Ayurveda & Allied sciences, central animal house, they were maintained on standard animal pellet feed & tap water was given ad-libitum. The temperature & humidity were kept at optimum & animals were exposed to natural day light cycles. The experiments were carried out in conformity with the institutional animal ethics committee & after obtaining its permission;

(Approval No: SDMCRA IAECI AM-H -10)

Equipments and glass ware:

Glass beakers and stirrer

Disposable syringes (2 ml).

Camera.

Infant feeding tube

Weighing Machine.

Hand gloves.

METHOD

EVALUATION OF HEPATOPROTECTIVE ACTIVITY

A number of experimental models are employed to assess, hepatoprotective activity. Paracetamol induced liver injury in rodent continues to be one among the most widely used models. Wagner demonstrated the anti- hepatoprotective effect of compound derived from extract of the white flowered varities of silybummarianum- silymarin which is generally taken as reference standard drug.

When rats are exposed to single doses of paracetamol, drug metabolites produce liver damage, this leads to changes in different types of parameters like ponderal, biochemical & histological. Administration of drug prior to toxicants administration will inhibit liver damage. This fact has been used to design experimental models for assessing antihepatotoxic or hepatoprotective activity.

In the present study hepatoprotective activity evaluation of Agniprabha vati was carried out using paracetamol induced hepatotoxicity model in rats.

PARACETAMOL INDUCED HEPATOTOXICITY

Hepatoprotective study using paracetamol induced hepatotoxicity model in rats was carried out using method followed by "An Investigation on Hepato-protective activity of Liv-Plus, a herbal formulation, against paracetamol& alcohol induced liver injury" M. Pharma (Ayu) Thesis, submitted to Gujarat Ayurveda University Jamanagar during 2009-10, by- Manish Ranjan, Guided by- Dr. B. Ravishankar, & Co-Guided- Dr. Ashok B.K174, with slight modifications by using oral route for inducing hepatotoxicity i.e., the paracetamol was administered through oral route.

DOSE FIXATION

The therapeutic dose of Agniprabha Vati- 1Masha i.e.,750 mg Daily dose of human beings)

Conversion formula- 750 * 0.018 = 13.5mg / 200. bd. wt.= 67.5mg / k.g . wt. Route of drug administration- orally

TREATMENT PROTOCOL:

- ✓ Group I: Normal control: animals received tap water.
- ✓ Group II: Positive control: animals received paracetamol (3g/kg, p.o.), and distilled Water.
- ✓ Group III: Standard drug treated: animals received Silymarin (70 mg/Kg, p.o.) in addition to paracetamol.
- ✓ Group IV: Test drug: animals received Agniprabha vati TED (67.5mg/kg, p.o.) with 20ml Kokilaksha kashaya in addition to paracetamol
- ✓ Group V: Test drug: animals received Agniprabha vati TED× 02-(135mg /kg,p.o.) with 20ml Kokilaksha kashaya in addition to paracetamol

The Test drug Agniprabha vati and reference drugs were administered orally for 6 consecutive days and one dose of the toxicant (Paracetamol) were administered orally to each group, except the water control group, on 6th day 1hr after test drug administration. The second dose of toxicant (paracetamol) were administered orally to each group except the water control group. After 48 hours of toxicant Paracetamol, the blood was collected in the tubes and sent for biochemistry laboratory for biochemical investigations. All the animals were sacrificed by cervical dislocation. Important organs like liver were dissected out, cleaned to remove extraneous tissues, blotted to remove blood stain and weighed. A piece of liver tissue was preserved in 10% formalin for histopathological processing.

Serum was separated and serum level of biochemical parameters namely SGOT, SGPT, TB, DB etc. were estimated

as per standard procedure prescribed by manufacturer (AGAPPE diagnostics Ltd., Kerala, India) whereas serum level of ALP was estimated as per standard procedure described by manufacturer (Span diagnostics Ltd., Surat, India) of diagnostic kit.

ASSESSMENT CRITERIA

Parameters employed for assessing the extent of liver Injury

✓ PONDERAL CHANGES

- *Body weight*- The change in the body weight was calculated for this purpose the body weight taken before & after the test drug administration was utilized.
- *Organ weight*- Liver weight was presented in terms of both absolute & relative weight

✓ SERUM BIOCHEMICAL PARAMETERS

The blood was collected, serum was separated & serum level of biochemical parameters namely SGOT, SGPT, Total Bilirubin, Direct Bilirubin & Serum level of alkaline phosphate etc, were estimated as per the standard procedure prescribed by manufacturer.

STATISTICAL ANALYSIS

The data obtained was analyzed by using modified 't' test & analysis of variance (ANOVA) followed by Dunnett's t test (GraphPad Instant version 3.5) for determining the level of significance of the observed effects.

All the values were expressed as mean ± SEM (Standard Error of Mean). A level of $P<0.05$ was considered as statistically significant and the value of $P<0.01$ or $P<0.001$ was considered statistically highly significant. Level of significance was noted and interpreted accordingly.

Percentage change:

The change of percentage is calculated by the formula

$$\%change = \frac{\text{final observation-initial observation X100}}{\text{Initial observation}}$$

II. PROCEDURE FOLLOWED TO PREPARE HISTOPATHOLOGICAL SLIDES

TISSUE PROCESSING

The tissues were placed in 10 % formalin for 24 to 48hrs for fixation

No	Processing solution	Time	Principle
1	70% Alcohol	30 minutes	Dehydration
2	90% Alcohol	30 minutes	
3	Absolute Alcohol I	1 hour	
4	Absolute Alcohol II	1 hour	
5	Absolute Alcohol III	1 hour	
6	Xylene I	45 minutes	Clearing
7	Xylene II	45 minutes	
8	Paraffin Wax I	30 minutes	
9	Paraffin Wax II	3-4 hours	Wax infiltration

Blocking or embedding the tissue was done to transfer the tissue from the final wax bath to a mould filled with molten paraffin wax. Thin sections of tissues block of 4 microns were cut with the help of microtome. The tissue sections were floated in water bath of temperature 50º- 52º and then taken in microscopic slides.

H & E STAINING

No	Staining solution	Time	Principle
1	Xylene I	5 minutes	Deparaffination
2	Xylene II	5 minutes	
3	Xylene III	10 minutes	
4	100% Alcohol	2 minutes	Rehydration
5	70% Alcohol	2 minutes	
6	50% Alcohol	2 minutes	
8	Running water	2 minutes	
8	Hematoxylin	2 minutes	
9	Running water	1 minutes	
10	Acid alcohol	2 dips	Differentiation
12	Running tap water	10 minutes	
13	Eosin	30-60 sec	
14	90% alcohol	1 dip	Dehydration
15	100% alcohol	2 mins	
16	Xylene	5mins	Clearing

Coverslip was placed in the slide and the prepared slides were seen under the LX-500 LED trinocular Research microscope (Labomed) and images were taken with MiaCam CMOS AR 6pro microscope camera connected to image AR pro software.

III. OBSERVATION & RESULTS OF HEPATOPROTECTIVE STUDY OF AGNIPRABHA VATI

PONDERAL CHANGES

Group	Body weight	% Change
Normal control	7.37±1.47	----
Positive control	0.18± 2.37	97%↓@
Standard group	13.64±1.99	7477.77↑#
Minimum test dose	-7.83±10.3	4,250↓#
Maximum test dose	6.14±2.37	3,311.11↑#

Data: Mean ±SEM
@-compared with normal control
#-compared with positive control
Table 01: Effect of Test drug Agniprabha vati on Body weight

The data shows there was decrease in body weight in positive control group when compared to the normal control. However the observed effects were statistically non-significant.

Standard drug i.e sylimarin and test formulation at double the therapeutic dose administered group showed increase in body weight in comparison to positive control group. The observed effects were statistically non-significant.

However the test drug administered at the therapeutic dose showed decrease level of body weight in comparison to positive control group and found to be statistically non-significant.

GROUP	liver weight (grams) Mean ±SEM	% Change
Normal control	9.53±0.44	----
Positive control	8.20±0.64	13.95↓@
Standard group	8.02±0.14	2.19↓#
Minimum test dose	7.16±0.65	12.68↓#
Maximum test dose	7.57±0.57	7.68↓#

Data: Mean ±SEM,
@-compared with normal control
#-compared with positive control

Table 2: Effect of Test drug Agniprabha vati on liver weight

The data shows there was decrease in liver weight in positive control group when compared to the normal control. However the observed effects were statistically non-significant.

Standard drug i.e sylimarin and test formulation at therapeutic dose and at double the therapeutic dose administered group showed decrease in liver weight in comparison to positive control group.

The observed effects were statistically non-significant.

SERUM BIOCHEMICAL PARAMETERS

Group	SGOT(U/L)	% Change
Normal control	136.16 ±6.17	----
Positve control	211.28± 22.8*	82.8↑@
Standard drug	177 ± 40.20	16↓#
Minimum test dose	142.6 ± 5.25	32↓#
Maximum test dose	145.2 ± 14.57	31.2↓#

*Data: Mean ±SEM, *P<0.05*
@-compared with normal control
#-compared with positive control

Table 3: Effect of test drug Agniprabha vati on serum SGOT

The data shows there was remarkable increase in serum SGOT level in positive control group when compared to the normal control.The observed changes were statistically significant.

Standard drug i.e. Sylimarin and Test formulation at therapeutic dose and at double the therapeutic dose administered groups showed decrease in the serum SGOT level in comparison to positive control group.

The observed effects were statistically non-significant.

Group	SGPT(U/L) Mean ±SEM	% Change
Normal control	72.5 ±8.45	-
Positive control	88.5 ± 8.08	22 ↑@
Standard drug	79.5± 6.43	10↓#
Minimum test dose	72.7 ± 4.4	17↓#
Maximum test dose	71.5 ± 2.71	19↓#

Data: Mean ±SEM, ns P>0.05
@-compared with normal control
#-compared with positive control

Table 4: Effect of test drug Agniprabha vati on serum SGPT

The data shows there was remarkable increase in serum SGPT level in positive control group when compared to the normal control. However the observed changes were statistically non-significant.

Standard drug i.e, sylimarin and test formulation at therapeutic dose and double the therapeutic dose administered groups showed decrease in serum SGPT level in comparison to positive control group.

The observed effects were statistically non-significant.

Group	ALP (U/L) Mean ± SEM	% Change
Normal control	678.6± 30.6	-
Positive control	426.5± 51.19**	37.1↓@
Standard drug	694 ±37.2**	62.7↑#
Minimum test dose	595.4 ± 91.06	39.6↑#
Maximum test dose	521.6 ± 43.7	22.2↑#

*Data: Mean ±SEM, ** P<0.0.1*
@-compared with normal control
#-compared with positive control

Table 5: Effect of test drug Agniprabha vati on serum ALP

The data shows there was decrease in ALP group when compared to the normal control

However the observed changes were statistically very significant.

Standard drug i.e, sylimarin showed increase in ALP level in comparison to positive control group. The observed effects were statistically very significant.

However the test formulation at therapeutic dose and at double the therapeutic dose administered showed elevated levels of ALP in comparison to positive control ground and was found to be statistically non- significant.

Group	Total protein Mean ±SEM	% Change
Normal control	6.47±0.24	-
Positive control	6.9±0.14	6.64↑ @
Standard drug	7.5±0.19	8.69↑#
Minimum test dose	7.5±0.32	8.69↑#
Maximum test dose	6.8±0.45	1.44↓#

Data: Mean ±SEM, ns P>0.05
@-compared with normal control
#-compared with positive control

Table 6: Effect of test drug Agniprabha vati on serum Total Protein

The data shows there was increase in total protein level in positive control group when compared to the normal control,

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however the observed changes were statistically non-significant.

Standard drug i.e, sylimarin and test formulation at therapeutic dose administered showed elevated levels of total protein in comparison to positive control group and was found to be statistically non-significant.

And test drug administered at double the therapeutic dose showed decrease level of Total protein in comparison to positive control group and was found to be statistically non-significant.

Group	Serum Albumin Mean ±SEM	% Change
Normal control	3.62±0.16	-
Positive control	3.06±0.14 *	15.46↓@
Standard drug	3.35±0.07	9.47↑#
Minimum test dose	3.53±0.27	15.35↑#
Maximum test dose	3.22±0.05	5.22↑#

*Data: Mean ±SEM, *P<0.0.5*
@-compared with normal control
#-compared with positive control
Table 7: Effect of test drug Agniprabha vati on serum Albumin

The data shows there was decrease in Serum albumin level in positive control group when compared to the normal control, however the observed changes were statistically significant.

Standard drug i.e, sylimarin and test formulation at therapeutic dose and test drug administered at double the therapeutic dose administered showed elevated levels of serum albumin in comparison to positive control group and was found to be statistically non-significant.

Group	Total bilirubin Mean ±SEM	% Change
Normal control	0.18±0.016	-
Positive control	0.168±0.012	6.66↓@
Standard drug	0.188±0.030	11.90 ↑#
Minimum test dose	0.173±0.02	2.97↑#
Maximum test dose	0.22±0.02	30.9↑#

Data : Mean ±SEM, ns P>0.05
@-compared with normal control
#-compared with positive control
Table 8: Effect of test drug Agniprabha vati on serum Total bilirubin

The data shows there was decrease in total bilirubin level in positive control group when compared to normal control group, however the observed changes were statistically non-significant.

Standard drug i.e, sylimarin , test formulation administered at therapeutic dose and at double the dose of therapeutic dose showed increase levels in total bilirubin level in comparison to positive control group. The observed effects were statistically non-significant.

Group	Direct bilirubin Mean ±SEM	% Change
Normal control	0.05±0.013	-
Positive control	0.07±0.010	40↑@
Standard drug	0.03±0.015	57↓#
Minimum test dose	0.05±0.004	28.5 ↓#
Maximum test dose	0.05±0.009	28.5 ↓#

Data: Mean ±SEM, ns P>0.05
@-compared with normal control
#-compared with positive control
Table 9: Effect of test drug Agniprabha vati on serum Direct bilirubin

The data shows there was increase in direct bilirubin in positive control group when compared to the normal control. However the observed effects were statistically non-significant.

Standard drug i.e sylimarin and test formulation at therapeutic dose and at double the therapeutic dose administered group showed decrease in direct bilirubin in comparison to positive control group. The observed effects were statistically non-significant.

IV. HISTOPATHOLOGICAL STUDY OBSERVATIONS

All the slides show liver tissue with lobular arrangement. Each lobules consists of a central vein and portal triads along the periphery of lobules. Numerous sinusoids pass radially from central vein and the spaces between the sinusoids contain liver cells.

Positive Control- The slides show diffuse cytoplasmic vasculation with degenerated cells showing enlarged and pale cytoplasm and clear vacuoles in most of the areas. Dilation of sinusoids are also seen. Mild chronic lymphocytic infiltrate is seen. Oedematous areas are also seen

Standard control- Mild cytoplasmic vasculation and dilation of sinusoids are seen in some areas. No inflammatory infiltrate. No oedema. Compared with positive control group, there is reduction in vasculation, reduced dilation of sinusoids and absence of inflammatory infiltrate.

Compared With Positive Control, Good Reduction In Histological Changes Is Seen

Low dose- Mild cytoplasmic vasculation and dilation of sinusoids are seen in some areas. Mild chronic inflammatory infiltrate is seen in most of the slides. Compared with positive control group, there is reduction in vasculation and reduced dilation of sinusoids

Compared With Positive Control, Reduction In Histological Changes Is Seen

High dose- Mild cytoplasmic vasculation and dilation of sinusoids are seen in some areas. Mild chronic inflammatory infiltrate is seen in most of the slides. Compared with positive control group, there is reduction in vasculation and reduced dilation of sinusoids

Compared With Positive Control, Reduction In Histological Changes Is Seen

SYMBOLS

Vascular degeneration, cytoplasmic vasculation- green arrow
Inflammatory infiltrate-red arrow
Dilated sinusoids-yellow arrow

Oedema-Black arrow

V. DISCUSSION

Non availability of specific drugs for treating different types of liver disorders is one of the major lacunas in the modern medicine. At present, the focus is on drugs used in different traditional system of medicine which ascribe hepatoprotective activity to a number of preparation based on natural products. There are several formulations with a claim of hepatoprotective activity in Indian market. Silymarin is being used for this purpose in an extensive manner. Nevertheless there is still scope for new addition to this category. Virus and alcohol induced liver injury is a major health problem. In addition recent years had shown increased incidence of drug induced hepatotoxicity as a potentially serious adverse effect. This situation demands addition of as many as possible good hepatoprotective formulations to the therapeutic use.

Among several models available for screening potential hepatoprotective drugs, paracetamol induced live injury is used quite often in recent times hence it was adopted for this study. To assess the hepatoprotective potential of the formulation *Agniprabha vati* in therapeutic dose (T1) and in double the dose of therapeutic dose (T2)-its effect was assessed on paracetamol induced changes in ponderal, biochemical and histopathological parameters. Simultaneously a group of animals received silymarin- a standard hepatoprotective drug for comparison purpose.

PONDERAL CHANGES

Parameters	Positive control	Standard grp	Minimum dose	Maximum dose
Body weight change	NSD	NSI	NSD	NSI
Liver weight change	NSD	NSD	NSD	NSD

Table 10: Effect of test drug on Ponderal changes

In positive control group and in minimum test dose group, there is non significant decrease in weight which may be suggestive of paracetamol metabolism affected reduction in the body weight. However, in standard and in Maximum test dose group there in non significant increase in weight is seen in failure of Paracetamol to affect body weight in this hepatotoxicity was parallel on the failure of thioacetamide to cause changes in body weight of rats in acute liver injury study.

There was no significant weight change was noted in liver, can be a sign of hepatoprotective activity, as it can indicate that a substance or treatment is protecting the liver

Parameters	Positive control	Standard group	Minimum dose	Maximum dose
SGOT	SI	NSD	NSD	NSD
SGPT	NSI	NSD	NSD	NSD
ALP	VSD	VSI	NSI	NSI
Total Protein	NSI	NSI	NSI	NSD
Albumin	SD	NSI	NSI	NSI
Total bilirubin	NSD	NSI	NSI	NSI
Direct bilirubin	NSI	NSD	NSD	NSD

NSD- Non significant decrease NSI- Non significant increase
SD- Significant decrease SI- Significant increase
VSD- Very significant decrease VSI- Very significant increase

Table 11: Effect of test drug on Serum biochemcial parameters

SGOT AND SGPT

Assesment of liver necrosis is done by estimation of SGOT (Serum Glutamic Oxaloacetic Transaminase), and SGPT stands for Serum Glutamate Pyruvate Transaminase.

TRANSMINASE ACTIVITY

It is well established that level of serum enzymes such as SGOT & SGPT gets elevated in paracetmol induced hepatotoxicity, which was very well noted in positive control group with elevated transminase activity indicative of liver inflammation & injury due to toxic effect of paracetamol, this elevation was significantly reversed by both test & standard groups indicative of hepatoprotective activity. Thus elevation in the transminase activity can be considered as an index of paracetamol induced hepatic toxicity & its reversal as sign of expression of hepatoprotection.

ALKALINE PHOSPHATASE

Serum alkaline phosphatase is produced by many tissues, especially bone, liver, intestine, and placenta and is excreted in the bile. Elevation in activity of this enzyme can thus be found in diseases of bone, liver and in pregnancy. In the absence of bone disease and pregnancy, an elevated serum alkaline phosphatase activity generally reflects hepatobiliary disease. ALPase is normally excreted in the bile. If it is affected due to liver injury its level gets elevated. This may be one of the reasons for the observed elevation since it is reported that greater elevation occurs in biliary tract obstruction.

In the present study, statistically very significant decrease in alkaline phosphatase activity was observed in positive control group. This was reversed by reference standard, TED and 2*TED doses of test drug. In fact very significant increase was observed at Standard grp and non significant increase in seen in TED and 2*TED grp.

TOTAL PROTEIN

Protein the building blocks of the body are in a state of dynamic equilibrium and are subjected to constant chemical attack. Changes in protein level are the favored mechanism for long term adaptive changes. In this study, positive control test drugs (TED1) and reference standard group produced non-significant increase in total protein level, and non significant decrease in positive control.

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This reveals the test drugs cytoprotective activity by potentiating the enzyme activities and altering metabolite flux and partitioning metabolites between different metabolic pathways. From the observed effect we assume that the test drug prevented the protein degradation which is characteristic of tissues undergoing major structural re-arrangement produced by the toxicant paracetamol. Again here it can be considered only as an indicator of the possible presence of hepatoprotection in the test formulation.

TOTAL ALBUMIN

Significantly decreased in Positive control suggests Liver disease, including severe cirrhosis, hepatitis, and fatty liver disease and Non significantly increased in test and standard group, suggestive of dehydration, indicating the removal of toxins by the liver.

TOTAL AND DIRECT BILIRUBIN

Bilirubin a break down product of haemoglobin is the predominant pigment produced in the liver, excess bilirubin causes yellowing of body tissues (Jaundice). There are two tests for bilirubin, direct- reacting (conjugated) and indirect-reacting (unconjugated).

Elevated levels of indirect bilirubin are usually caused by liver cell dysfunction (eg. hepatitis), while elevation of direct bilirubin typically result from obstruction either within the liver (intra- hepatic) or source outside the liver (eg. Gall stone or tumor). In the present study serum bilirubin was non significantly decreased in positive control suggestive of hepatoprotective activity Increase in serum bilirubin level in test and standard groups was again suggestive of liver cell dysfunction.

Direct bilirubin is non significantly increased in positive control, suggestive of Liver cell dysfunction and non significantly decreased levels suggests the hepatoprotective activity of test and standard drugs.

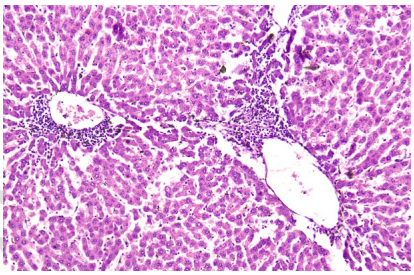
VI. CONCLUSION

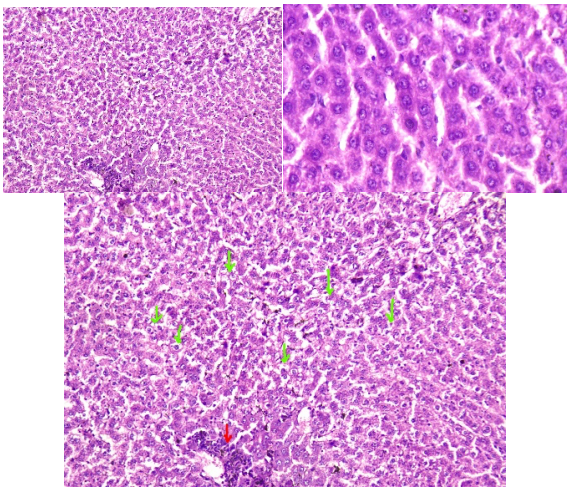
- ✓ Experimental study was carried out in order to evaluate Hepato-protective acitivity of Agniprabha vati on wistar albino rats.
- ✓ The overall activity profile indicated reversal of important parameters like SGOT, SGPT, ALP, serum total cholesterol and serum bilirubin changes.
- ✓ In sylimarin group good protection was observed, though moderate injury was observed in 2 rats-because of this sylimarin did not effectively reverse the biochemical parameter changes.
- ✓ Histopathological results indicate moderate to good protection was observed in TED group- co-relating very well with the changes observed in the biochemical parameters.
- ✓ TED dose group of test drug possess significant hepato-protective activity in all the parameters.
- ✓ TEDX2 dose level showed significant hepato-protective activity in all parameters.
- ✓ Hence, the activity is not dose dependent.

The findings of the present study demonstrate that Agniprabha Vati possesses significant hepatoprotective potential, comparable to silymarin in a paracetamol-induced hepatotoxicity model in rats. The formulation normalized liver enzymes, improved serum proteins, and preserved hepatic architecture. While some results were statistically non-significant, the overall trend favoured hepatoprotection. These findings scientifically validate the classical usage of Agniprabha Vati in Yakrit Vikara and encourage further research into its clinical applications. Future studies may focus on long-term toxicity, pharmacokinetics, and human clinical trials to establish it as a standard hepatoprotective agent in integrative medicine.

HISTOPATHOLOGY PICTURES

High dose





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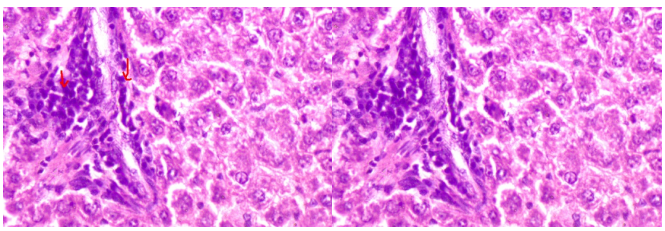

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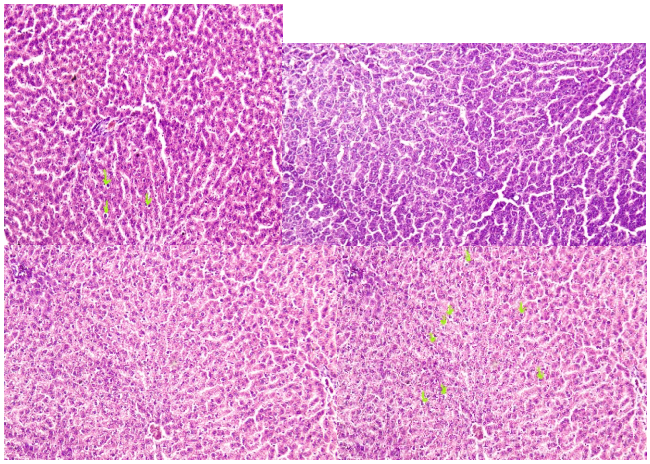
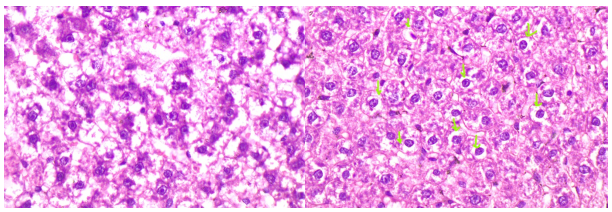
Standard dose



Positive control



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3	Effect of test drug Agniprabha vati on serum SGOT.
4	Effect of test drug Agniprabha vati on serum SGPT
5	Effect of test drug Agniprabha vati on serum ALP.
6	Effect of test drug Agniprabha vati on serum Total Protein.
7	Effect of test drug Agniprabha vati on serum Albumin.
8	Effect of test drug Agniprabha vati on Total Bilirubin.
9	Effect of test drug Agniprabha vati on Direct Bilirubin.
10	Effect of test drug on Ponderal changes
11	Effect of test drug on Serum biochemical parameters

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