Research Article

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HEPATOPROTECTIVE EFFECT OF POLYHERBAL FORMULATIONS ON PARACETAMOL INDUCED LIVER TOXICITY IN RATS

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ABSTRACT

The study was carried out to evaluate the hepatoprotective effect of polyherbal formulation YAK samples on paracetamol induced liver toxicity in rats. Albino rats were divided into six groups consisting of twelve animals in each group. Each group was subdivided into preventive & curative groups. Group I served as Healthy control. Group II received paracetamol (2 gm/kg, p.o. on 13th day), Groups III, IV, V and VI received Silymarin, YAK-001, YAK-PVX002 and YAK-PVZ003 respectively orally for 15 days. On 13th day 6 animals in each group were administered paracetamol (2 gm/kg, p.o) After 48 hours of paracetamol administration, these rats were subjected for preventive effect evaluation. Similarly, the animals in all the curative groups received a single dose of paracetamol (2 gm/kg, p.o.) on the 1st day followed by the respective drug treatment as in preventive group for 15 days to evaluate the curative effect. Serum biomarker levels were assessed in serum and histopathology of liver was assessed. Significant ($p<0.01$) rise in serum levels of SGOT, SGPT, ALP, GGT and ACP was observed in paracetamol (2 gm/kg, p.o.) treated rats. In contrast, treatment with YAK-001, YAK-PVX002 and YAK-PVZ003 exhibited the ability to counteract the paracetamol induced hepatotoxicity by decreasing the serum enzyme levels when compared to control in preventive and curative studies. YAK-001 showed enhanced preventive and curative effect in hepatotoxicity induced in rats than YAK-PVX002 and YAK-PVZ003 in albino rats. However, curative effect is enhanced hepatoprotection than preventive effect.

Keywords: Polyherbal, YAK samples, Paracetamol, Hepatoprotective

INTRODUCTION

Liver diseases remain one of the serious health problems. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders in ethno medical practices as well as in traditional systems of medicine in India.[1] Liver is a key organ regulating homeostasis within the body by various functions. Liver injury caused by toxic chemicals and certain drugs has been recognized as a toxicological problem.[2] Hepatotoxicity is one of very common aliment resulting into serious weakness ranging from severe metabolic disorders to even mortality.[3] Medicinal plants have very important role in the health of human beings as well as animals. As per the WHO estimates, about 80% of the world's population currently use herbs and other traditional medicines to cure various diseases, including liver disorders.[4] Several phytomedicines are nowadays used for the prevention and treatment of various liver disorders. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. The Indian traditional medicine like Ayurveda, Siddha and Unani is estimated that about 7500 plants are used in health traditions out of these, the real medicinal value of over 4000 plants is either little known or unknown to the mainstream population.[5] In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cell. Many formulations containing herbal extracts are sold in the Indian market for liver disorders. But management of liver disorders by a simple and precise herbal drug is still an exciting problem. Several Indian medicinal plants have been extensively used in the Indian traditional system of medicine for the management of liver disorder.[6] The present study was planned to investigate 3 polyherbal formulations YAK samples on paracetamol induced hepatotoxicity in rats. A single dose acute oral toxicity study was done on YAK-001 by employing OECD guidelines 425 and the test drug did not show any toxic potential even at the dose of 2000 mg/kg.[7]

MATERIALS & METHODS

Drugs & Chemicals

Silymarin- Sigma Aldrich, Bangalore, India. Estimation kits-Swemed Diagnostics, Bangalore, India. YAK samples were procured from Sri Sri Ayurveda Trust, Bangalore, India. All other chemicals were obtained (Himedia, Bangalore, India) were of analytical grade.

Test Samples

All the samples are poyherbal formulations. Dry herbs are powdered into fine powders and passed through #80 mesh and mixed homogenously in different combinations. Process is carried out in hygienic condition and samples are stored into air tight containers.

Animals

Albino wistar rats (180–220 g) were procured from Sri Ragavendra Enterprises, Bangalore, Karnataka, India, and used throughout the study. They were housed in polypropylene cages in a controlled environment (temperature 25±2ºC and 12 h dark/light cycle) with standard laboratory diet and water *ad libitum*. The protocol of hepatoprotective activity (IAEC/ABMRCP/2014-2015/12) was approved by the Institutional Animal Ethical Committee of Acharya & B.M.

Reddy College of Pharmacy, Soldevanahalli, Bangalore, Karnataka, as per the guidelines of CPCSEA.

Experimental Design
The Albino wistar rats were randomly divided into six groups of twelve animals each after weighing, recording and numbering. Each group was further subdivided into Preventive & Curative groups which received the following treatment:

Preventive Study
Group-I: Healthy control
Group-II: Paracetamol (2 gm/kg, p.o. on 13th day)
Group-III: Paracetamol (2 gm/kg, p.o. on 13th day) + Standard drug (Silymarin, 50 mg/kg, p.o)
Group-IV: Paracetamol (2 gm/kg, p.o. on 13th day) + YAK-001 for 15 days
Group-V: Paracetamol (2 gm/kg, p.o. on 13th day) + YAK-PVX002 for 15 days
Group-VI: Paracetamol (2 gm/kg, p.o. on 13th day) + YAK-PVZ003 for 15 days
Group I had received a single dose of 1.5 ml of 2 % gum acacia (healthy control) orally. Group II had received paracetamol (2 gm/kg, p.o. on 13th day), Group III had received the standard drug (Silymarin suspension in doses of 50 mg/kg, for 13 days). Groups IV, V and VI had received a single dose of test drug YAK-001, YAK-PVX002 and YAK-PVZ003 respectively orally for 15 days. On 13th day all the groups were treated with paracetamol (2 gm/kg, p.o). After 48 hours of paracetamol administration, only six rats from all the groups were subjected for evaluation (biochemical estimation and histopathology) to see the preventive effect.

Curative Study
Group-I: Healthy control
Group-II: Paracetamol (2 gm/kg, p.o. on 1st day)
Group-III: Paracetamol (2 gm/kg, p.o. on 1st day) + Standard drug (Silymarin, 50 mg/kg, p.o)
Group-IV: Paracetamol (2 gm/kg, p.o. on 1st day) + YAK-001 for 15 days
Group-V: Paracetamol (2 gm/kg, p.o. on 1st day) + YAK-PVX002 for 15 days
Group-VI: Paracetamol (2 gm/kg, p.o. on 1st day) + YAK-PVZ003 for 15 days
For remaining six animals in all the groups, drug treatment was begun orally after a single dose of paracetamol (2 gm/kg, p.o.) on the 1st day followed by administration of trial drugs for subsequent 15 days to evaluate the curative effect.[8]

Dosage of trial drugs: The dosage of all the 3 trial drugs YAK-001, YAK-PVX002, YAK-PVZ003 were 405mg /Kg p.o which was fixed based on the Ayurvedic dosage form as advised to be efficacious as per the Research & Development division, Sri Sri Ayurveda Trust, Bangalore.

Biochemical evaluation
Blood (2 ml) was collected from rats after the last dose of the drug from retro- orbital sinus plexus under mild ether anaesthesia and allowed to clot for 30 minutes. Serum were separated by centrifugation at 2,500 rpm at 30°C for 15 min and used for analyses of liver function test-serum such as SGPT, SGOT. Direct & Total Bilirubin, GGT, AMP & ACP and lipid profiles.[9] The rats were sacrificed by cervical dislocation and the liver was isolated. The liver were quickly excised and perfused with chilled normal saline to completely remove all the blood cells and subjected for liver function test (SGOT, SGPT and ALP only). A part of the liver was stored in 10% formalin for histopathological examination.

Histopathology of liver
A fresh piece of the liver from each rat, previously trimmed to approximately 2 mm thickness, was rapidly fixed in 10 % neutral formalin. The fixed tissues were then embedded in paraffin, sectioned (5 µm) with a rotary microtome and stained with haematoxylin and eosin (H&E). The liver sections were evaluated histologically with a camera attached to a light microscope (Nikon E400).[10]

Statistical evaluation
Data were expressed as mean ± standard error of mean. Statistical comparisons were made by using one-way ANOVA followed by Dunnet's multiple comparison test. The results were considered statistically significant if $P < 0.05$.

RESULTS & DISCUSSION

Preventive study
There was significant ($p<0.01$) rise in serum of SGOT, SGPT, ALP, GGT and ACP after administration of paracetamol (2 gm/kg, p.o.) in pre-treated animals. In contrast, treatment with YAK-001, YAK-PVX002 and YAK-PVZ003 exhibited the ability to counteract the paracetamol induced hepatotoxicity by decreasing the serum enzymes levels ($p<0.01$) when compared to control.
YAK-001 at a dose of (405 mg/kg, p.o.) showed a percentage protection of 93.16, 97.82, 69.48, 85.84 & 61.92 for SGOT, SGPT, ALP, GGT and ACP respectively. Silymarin at a dose of (50 mg/kg, p.o.) showed a percentage protection of 81.75, 92.55, 83.62, 98.23 and 71.64 for SGOT, SGPT, ALP, GGT and ACP respectively. YAK-PVX002 at a dose of (405 mg/kg, p.o.) showed a percentage protection of 37.96, 51.9, 26.72, 42.65 and 27.07 for SGOT, SGPT, ALP, GGT and ACP respectively. YAK-PVZ003 at a dose of (405 mg/kg, p.o.) showed a percentage protection of 43.51, 62.78, 66.81, 71.68 and 42.06 for SGOT, SGPT, ALP, GGT and ACP respectively.

Curative study
There was significant ($p<0.01$) rise in serum of SGOT, SGPT, ALP, GGT and ACP after administration of paracetamol (2 gm/kg, p.o.) in post-treated animals. In contrast, treatment with YAK-001, YAK-PVX002 and YAK-PVZ003 exhibited the ability to counteract the paracetamol induced hepatotoxicity by decreasing the serum enzymes levels ($p<0.01$) when compared to control.
YAK-001 at a dose of (405 mg/kg, p.o.) showed a percentage protection of 93.33, 97.42, 76.60, 93.62 and 74.19 for SGOT, SGPT, ALP, GGT and ACP respectively. Silymarin at a dose of (50 mg/kg, p.o.) showed a percentage protection of 91.22, 95.58, 91.56, 99.73 and 81.71 for SGOT, SGPT, ALP, GGT and ACP respectively. YAK-PVX002 at a dose of (405 mg/kg, p.o.) showed a percentage protection of 56.73, 59.03, 33.63, 59.70 and 45.71 for SGOT, SGPT, ALP, GGT and ACP respectively. YAK-PVZ003 at a dose of (405 mg/kg, p.o.) showed a percentage protection of 70.42, 76.02, 74.67, 84.26 and 65.58 for SGOT, SGPT, ALP, GGT and ACP respectively.

Histo-pathological analysis
The extent of paracetamol-induced liver damage was evaluated based on histopathologic studies also supported the evidence of biochemical analysis. In preventive, liver parenchyma was normal in healthy control. Paracetamol induced group showed increased centrilobular necrosis (perivenular necrosis) along with degenerative changes in the midzonal hepatocytes (cytoplasmic vacuolations). The periportal hepatocytes appeared normal with some congested central veins. Silymarin group showed normal hepatocytes like healthy control. With YAK-

001, the architecture of liver was restored and the necrotic hepatocytes were minimal almost appearing like silymarin group. YAK-PVX002 group showed increased centrilobular necrosis of hepatocytes like that of paracetamol group. The morphological changes in YAK-PVZ003 hepatocytes were in between YAK-001 and YAK-PVX002 groups.

But in curative, Liver parenchyma was normal in healthy control. Paracetamol induced group showed increased degenerative changes in all zones of hepatocytes (cytoplasmic vacuolations). The periportal hepatocytes showed dense chronic inflammatory infiltration along with some congested central veins. Silymarin group showed periportal inflammatory aggregates with dilated and congested sinusoids. With YAK-001, the hepatocytes appeared like healthy control. YAK-PVX002 group showed increased degenerative hepatocytes like paracetamol group. The morphological changes in YAK-PVZ003 hepatocytes were in between YAK-001 and YAK-PVX002 groups.

Histological examination of rat liver treated with paracetamol shows significant hepatotoxicity characterized by necrosis of hepatocytes and congested of the central veins. There was extensive infiltration of the lymphocytes and loss of cellular boundaries. However, in animals treated with YAK-001, YAK-PVX002 and YAK-PVZ003 samples the severity of hepatic damage was decreased when compared with the hepatic damage observed in paracetamol treated. Administration of YAK-001 significant reduced the hypertrophy of hepatocytes and lymphocyte infiltration in the central vein was decreased, which further indicated its significant hepatoprotective effect in liver sections stained with H&E method.

Table 1: Effects of YAK-001, YAK-PVX002 and YAK-PVZ003 on liver biochemical parameters in paracetamol induced hepatotoxicity in rats (Preventive)

GROUP	SGOT (U/I)	SGPT (U/I)	ALP (U/I)	GGT (U/I)	ACP (U/I)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	Direct Bilurubin (mg/dl)	Total Bilurubin (mg/dl)
Healthy control	128.80 ± 1.7	92.80 ±2.13	257.00 ± 2.1	6.60 ± 0.22	32.84 ±2.40	45.00 ± 2.19	32.00 ± 2.7	41.75 ± 2.1	0.15 ± 0.01	0.42 ± 0.02
Paracetamol (2gm/kg, p.o.)	260.51 ± 1.6	285.75 ± 2.5	373.00 ± 1.4	12.25 ± 0.24	58.66 ± 3.26	74.50 ± 2.4	70.83 ± 2.8	29.34 ± 2.16	0.28 ± 0.01	0.65 ± 0.04
Silymarin (50 mg/kg, p.o.)	152.83 ± 3.8**	107.17 ± 3.8**	276.00 ± 2.09**	6.70 ± 0.15**	40.16 ±2.63**	51.20± 1.87**	47.50 ± 3.3**	36.23 ± 3.6**	0.18 ± 0.02**	0.43 ± 0.04**
YAK-001 (405 mg/kg, p.o.)	137.80 ± 1.1**	97.00 ± 1.7**	292.40 ± 3.2**	7.40 ± 0.13**	42.67 ± 2.58**	48.32 ± 3.14**	51.60 ± 2.5**	34.76 ± 2.5*	0.17 ± 0.02**	0.36 ± 0.02**
YAK-PVX002 (405 mg/kg, p.o.)	210.50 ± 1.6**	185.60 ± 3.2**	342.00 ± 2.7**	9.84 ± 0.16**	51.67 ± .67**	67.50 ± 3.5**	68.17 ± 3.1^{ns}	31.34 ± 3.3 ^{ns}	0.22 ± 0.02**	0.52 ± 0.03**
YAK-PVZ003 (405 mg/kg, p.o.)	203.20 ± 1.8**	164.60 ± 2.1**	295.50 ± 1.8**	8.20 ± 0.14**	47.8 ± 3.70**	61.80 ± 3.7**	63.40 ± 3.2**	32.25 ± 2.9 ^{ns}	0.21 ± 0.01**	0.48 ± 0.02**
All values are expressed in Mean ± S.D; the results were analysed using Prism, version-5. One way analysis of variance (ANOVA) test followed by Dunnett's post hoc test was used to analyse the results, * p< 0.05, ** p< 0.01 was considered as statistically significant.										

Table 2: Effects of YAK-001, YAK-PVX002 and YAK-PVZ003 on tissue parameters in paracetamol induced hepatotoxicity in rats (Preventive)

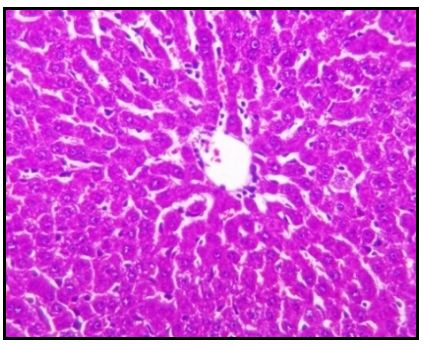
GROUP	On 15th day (Preventive)		
	SGOT (U/I)	SGPT (U/I)	ALP (U/I)
Healthy control	4.94 ± 0.75	6.64 ± 0.57	65.43 ± 3.51
Paracetamol (2gm/kg, p.o.)	9.02 ± 0.73	11.00 ± 0.81	102.19 ± 2.74
Silymarin (50 mg/kg,p.o.)	5.63 ± 0.56**	6.80 ± 0.69**	73.40 ± 3.35**
YAK-001 (405 mg/kg,p.o.)	6.27 ± 0.47**	7.52 ± 0.60**	75.63 ± 3.60**
YAK-PVX002 (405 mg/kg,p.o.)	8.40 ± 0.61**	10.14 ± 0.87^{ns}	92.40 ± 4.72**
YAK-PVZ003 (405 mg/kg,p.o.)	7.57 ± 0.80**	8.22 ± 0.77**	80.71 ± 3.46**
All values are expressed in Mean ± S.D; the results were analysed using Prism, version-5. One way analysis of variance (ANOVA) test followed by Dunnett's post hoc test was used to analyse the results, * p< 0.05, ** p< 0.01 was considered as statistically significant.			

Table 3: Effects of YAK-001, YAK-PVX002 and YAK-PVZ003 on liver biochemical parameters in paracetamol induced hepatotoxicity in rats (Curative)

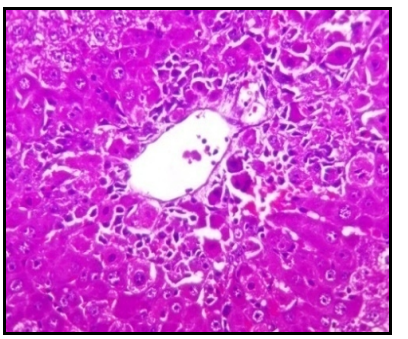
GROUP	SGOT (U/I)	SGPT (U/I)	ALP (U/I)	GGT (U/I)	ACP (U/I)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	Direct Bilurubin (mg/dl)	Total Bilurubin (mg/dl)
Healthy control	127.34 ± 2.33	94.5 ± 2.58	255.17 ± 2.63	6.78 ± 0.21	31.83 ± 2.31	42 ± 1.89	32.84 ± 2.04	40.5 ± 1.51	0.14 ± 0.014	0.41 ± 0.16
Paracetamol (2gm/kg, p.o.)	277.17 ± 3.06	294.66 ± 3.26	367.67 ± 2.80	14.15 ± 0.28	62.83 ± 3.18	80.17 ± 2.48	78.5 ± 1.87	25.16 ± 1.47	0.29 ± 0.024	0.88 ± 0.023
Silymarin (50 mg/kg,p.o.)	140.5 ± 2.58**	103.34 ±3.07**	264.67 ± 3.93**	6.8 ± 0.80**	37.5 ± 2.58**	48.5 ± 2.25**	42.83 ± 1.16**	39.5 ±1.87**	0.16 ± 0.014**	0.42 ± 0.023**
YAK-001 (405 mg/kg,p.o.)	137.34 ± 1.21**	99.67± 2.33**	281.5± 4.13**	7.25 ± 0.32**	39.83 ± 2.78**	47.83 ± 2.63**	48.66 ± 1.86**	36.83 ±1.16**	0.16 0.02**	0.4 ± 0.02**
YAK-PVX002 (405 mg/kg,p.o.)	192.17 ± 3.71**	176.5± 3.68**	329.84 ± 4.21**	9.75 ± 0.50**	48.66 ± 2.65**	61.34 ± 1.75**	62.84 ± 2.63**	32.67 ±2.16**	0.2 ± 0.014**	0.5 ± 0.023**
YAK-PVZ003 (405 mg/kg,p.o.)	171.66 ±3.98**	142.5± 3.50**	283.67 ±4.41**	7.94 ± 0.20**	42.5 ± 3.27**	55.66 ± 2.73**	57.17 ± 2.85**	35.00 ±3.28**	0.19 ± 0.23**	0.46 ± 0.050**
All values are expressed in Mean ± S.D; the results were analysed using Prism, version-5. One way analysis of variance (ANOVA) test followed by Dunnett's post hoc test was used to analyse the results, * p< 0.05, ** p< 0.01 was considered as statistically significant.										

Table 4: Effects of YAK-001, YAK-PVX002 and YAK-PVZ003 on tissue parameters in paracetamol induced hepatotoxicity in rats (Curative)

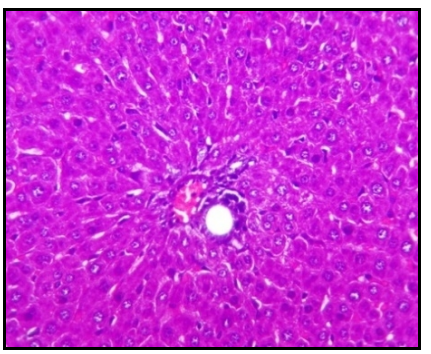
GROUP	On 30th day (Curative)		
	SGOT (U/I)	SGPT (U/I)	ALP (U/I)
Healthy control	4.76 ± 0.35	6.66 ± 0.57	65.37 ± 3.10
Paracetamol (2gm/kg, p.o.)	11.24 ± 0.75	14.21 ± 0.46	105.71 ± 1.96
Silymarin (50 mg/kg,p.o.)	4.93 ± 0.22**	6.72 ± 0.34**	69.72 ± 1.62**
YAK-001(405 mg/kg,p.o.)	5.73 ± 0.31**	7.16 ± 0.33**	70.49 ± 2.37**
YAK-PVX002 (405 mg/kg,p.o.)	7.28 ± 0.27**	9.67 ± 0.39**	89.57 ± 2.78**
YAK-PVZ003 (405 mg/kg,p.o.)	6.86 ± 0.38**	7.95 ± 0.37**	77.46 ± 2.35**
All values are expressed in Mean ± S.D; the results were analysed using Prism, version-5. One way analysis of variance (ANOVA) test followed by Dunnett's post hoc test was used to analyse the results, * p< 0.05, ** p< 0.01 was considered as statistically significant.			



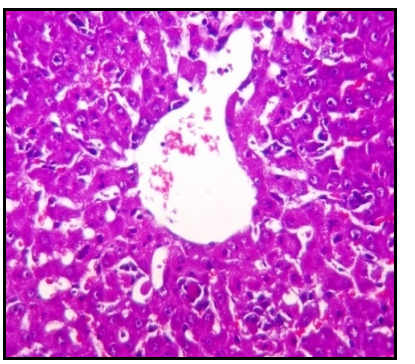
A: Healthy control, shows intact architecture. The perivenular hepatocytes, periportal hepatocytes and midzonal hepatocytes & periportal region appear unremarkable.



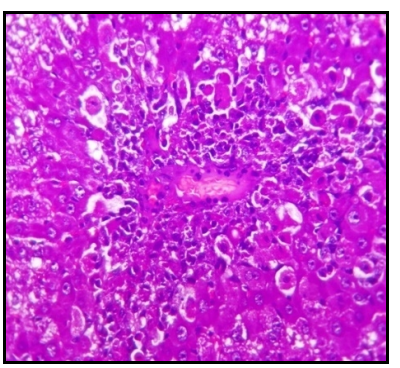
B: Positive control, liver parenchyma partially disrupted, extensive necrosis with inflammation. Midzonal hepatocytes show mild cytoplasmic vacuolations & some of central veins appear congested.



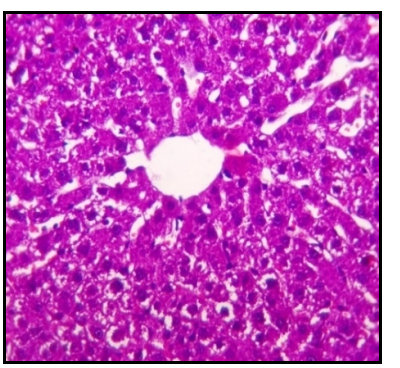
C: Silymarin treated, shows intact structure.



D: YAK-001 treated, Few scattered perivenular hepatocytes show necrotic changes.

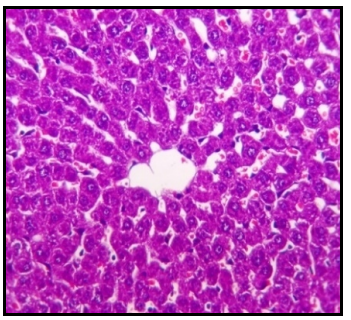


E: YAK-PVX002 treated, Most of the perivenular hepatocytes show extensive necrosis along with dense inflammatory infiltration. The midzonal hepatocytes show mild cytoplasmic vacuolations. Some of the central veins appear dilated and congested.

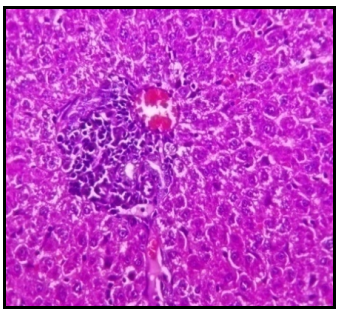


F:YAK-PVZ003 Some of the perivenular hepatocytes appear necrotic while most of the perivenular hepatocytes. Midzonal hepatocytes show cytoplasmic vacuolations. The periportal region shows mild inflammatory infiltration.

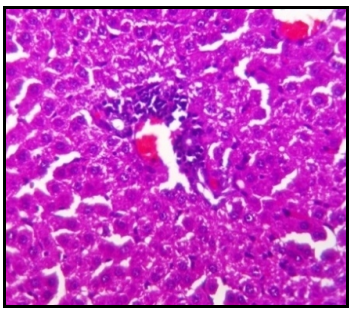
Figure 1: Histological sections of preventive study



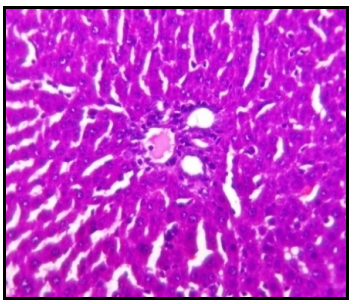
A, healthy control, intact architecture. The perivenular hepatocytes, periportal hepatocytes, midzonal hepatocytes, periportal region,liver parenchyma, central veins and sinusoids were unremarkable.



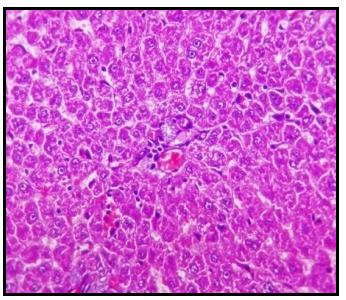
B. Positive control, intact architecture. Most of the perivenular hepatocytes, midzonal hepatocytes and periportal hepatocytes show cytoplasmic vacuolations.The periportal region shows dense aggregates of chronic inflammatory infiltration. Most of the central veins appear congested.



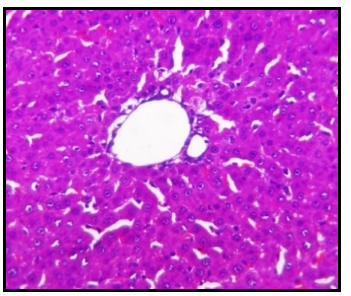
C: Silymarin treated, liver parenchyma shows intact architecture. The perivenular hepatocytes and midzonal hepatocytes appear unremarkable. The periportal region shows dense aggregates of chronic inflammatory infiltration. Some of the sinusoids are dilated and congested.



D: YAK-001 treated, The perivenular hepatocytes, periportal hepatocytes, midzonal hepatocytes, periportal region,liver parenchyma, central veins and sinusoids were unremarkable. Liver parenchyma shows intact architecture.



E: YAK-PVX002 treated, The periportal region shows scant chronic inflammatory infiltration. Most of the sinusoids are dilated and congested. Liver parenchyma shows intact architecture. Most of the perivenular hepatocytes, midzonal hepatocytes and periportal hepatocytes show cytoplasmic vacuolations.



F: YAK-PVZ003 treated, liver parenchyma shows intact architecture. The perivenular hepatocytes and midzonal hepatocytes appear unremarkable. Few of the periportal hepatocytes show cytoplasmic vacuolations. The periportal region shows scant chronic inflammatory infiltration. Most of the sinusoids are dilated and congested

Figure 2: Histological sections of curative study

DISCUSSION

A liver injury induced by paracetamol is one of the mainly characterized system of xenobiotic induced hepatotoxicity and commonly used model for the screening of hepatoprotective activity of drugs[11]. Silymarin is a polyphenolic flavonoid isolated from the fruit and seeds of the milk thistle (Silybum marianum)[12]. Various studies indicate that silymarin exhibits strong antioxidant activity[13] and shows protective effects against hepatic toxicity induced by a wide variety of agents by inhibiting lipid peroxidation[14-15],. Hepatotoxic drugs, such as paracetamol, are known to cause marked elevation in serum level of enzymes, such as SGOT, SGPT, ALP, GGT, ACP and bilirubin, indicating significant hepatocellular injury[16]. Raised activity of serum transaminases in intoxicated rats, as observed in the present study, can be attributed to the damaged structural integrity of the liver because these are cytoplasmic in nature and are released into the circulation after cellular damage. Observed in rats treated with paracetamol and may be associated with the decrease in the number of hepatocytes, which in turn may result in the decreased hepatic capacity to synthesize protein and consequently decrease liver weight.

To the best of our knowledge, we report that administration of YAK-001 ameliorated paracetamol induced acute liver injury in rats, as evidenced by both histological and biochemical findings. Similar protective effects were also observed in rats receiving silymarin, which was used as a positive control. YAK-001, comprises chiefly of Ayurvedic herbs Bhumyamalaki (*Phyllanthus niruri*), Katuki (*Picrorhiza kurroa*), Bhunimba (*Andrographis paniculata*), Sharapunkha (*Tephrosia purpurea*), Patola (*Trichosanthes dioica*), Punarnava (*Boerhavia diffusa*), Bhringaraja (*Eclipta alba*) etc., which are known to have proven

effects in combating liver disorders. *Phyllanthus niruri* has anti-oxidant and hepato-protective activity against CCl_4 induced hepatotoxic rats with associated deleterious effects on kidney and testes.[17] *Picrorhiza kurroa* extract brought about a reversal of the fatty infiltration of the liver (mg/g) and a lowering of the quantity of hepatic lipids.[18] *Andrographis paniculata* possesses significant hepatoprotective activity aginst CCl_4 induced hepatotoxicity in rats.[19] *Tephrosia purpurea* showed that supplementation of extract (500 mg/kg) could ameliorate the hepatotoxic action of arsenic.[20] *Trichosanthes dioica* Roxb has significant hepatoprotective activity paracetamol induced hepatic damage in rats.[1] The roots of *Boerhaavia diffusa* L., commonly known as '*Punarnava'*, are used by a large number of tribes in India for the treatment of various hepatic disorders.[21] *Eclipta alba* extract showed hepatoprotective effect against paracetamol induced hepatic damage in mice.[22] These studies are supportive to indicate YAK-001 which has been chiefly made out of these herbs has shown significant results.

CONCLUSION

The present study results indicates that YAK-001 shows the maximum curative effect in hepatotoxicity induced in rats than Silymarin, YAK-PVX002 and YAK-PVZ003 by decreasing elevated hepatic enzymes, biochemical parameters of serum and liver in albino rats. However, curative effect is enhanced hepatoprotection than preventive effect by decreasing biochemical markers, hepatic injury and hepatic toxicity.

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