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Research Article

HEPATOPROTECTIVE ACTIVITY OF YAKRIT PLIHANTAK CHURNA AGAINST CARBON TETRACHLORIDE INDUCED OXIDATIVE STRESS AND HEPATOTOXICITY IN WISTAR ALBINO RATS

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ABSTRACT

The objective of this study is to evaluate the hepatoprotective effect of the Yakrit plihantak churna against CCl_4 induced hepatotoxicity in Wistar albino rats. The methods are hepatoprotective activity was assessed using CCl_4 induced hepatic injury in rats by monitoring the biochemical parameters along with oxidative stress markers. Biochemical markers of hepatic injury such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH). The liver function was assessed by monitoring the level of total bilirubin, total protein and levels of albumin in serum. The oxidative stress was evaluated by measuring the levels of Malondialdehyde and superoxide dismutase. The results are CCl_4 and olive oil mixture of (1:1 dosage of 1 ml/kg b.w) induced oxidative stress was indicated by elevated levels of AST, ALT, ALP, LDH, total bilirubin and MDA. The depleted levels of total protein, albumin and superoxide dismutase were found in CCl_4 induced animals. The Yakrit plihantak churna at two dose levels 200 mg/kg and 400 mg/kg significantly re-established the hepatic parameters towards normal level as compared to the elevated biochemical markers enzymes in the CCl_4 treated animals. Reversal to normal tissue architecture was observed in histological evaluation. The results of YPC (400 mg/kg) were found comparable with that of the standard drug silymarin in all the parameters. The conclusion is the above findings suggest the therapeutic potential of these plants in alleviating hepatic oxidative stress and tissue damage. This shows its hepatoprotective activity. Further *in-vivo* studies are needed to explore the possible mechanism of action of isolated compounds derived from Yakrit plihantak churna in treating liver diseases.

Keywords: Hepatoprotective, poly herbal formulation, oxidative stress, hepatotoxicity.

INTRODUCTION

The liver is an organ with a broad set of critical biologic functions, a unique dual vascular supply, and several distinct cell types that contribute to its physiologic functions as well as potential pathology.[1] Because of its unique metabolic function and relationship to the gastrointestinal tract, liver is an important target of toxicity to xenobiotics, oxidative stress, ethanol and toxic chemicals.[2] Large amount of free radicals are generated by various toxins as well as by xenobiotic pathways of liver cause hepatic injury by the mechanism of covalent binding and lipid peroxidation and these reactive species contribute largely to the liver injury. Oxidative stress is viewed as an imbalance between the production of reactive oxidant species (ROS) and their elimination by protective mechanisms, which can lead to chronic inflammation. The inflammation triggered by oxidative stress is the cause of liver injury which later advances to serious chronic conditions.[3]

Oxidative stress- the primary phase of liver injury, when exceeds beyond the protective capacity of anti oxidative mechanisms of the body, ultimately leads to the liver damage and onset of chronic diseases associated with the liver. Carbon tetrachloride (CCl_4) is one of the commonly used hepatotoxin that induces liver damage in experimental systems.[4-6]

Liver damage after the administration of CCl_4 is believed to be effected by the trichloro methyl radical ($^{*}CCl_3$) that adversely affects membrane lipids, proteins and nucleic acids.[7] Oxidative stress has been considered as a conjoint pathological mechanism, and it contributes to initiation and progression of liver injury so application of antioxidants signifies a rational curative strategy to prevent and cure liver diseases involving oxidative stress.

Natural antioxidants and hepatoprotective agents are widely accepted over conventional drugs with drastic side effects to be used in mitigating oxidative stress and further advancement into liver damage. Recently, many folk remedies from plant origin are being evaluated for its possible antioxidant and hepatoprotective effects against different chemical induced liver damage in experimental animals.[8,9] Many herbals are on market to support health, relieve symptoms and cure diseases. However, most of these products lack scientific evidence that supports these allegations. One such product is Yakrit plihantak churna a poly herbal formulation consisting of plant parts of *Phyllanthus niruri, Picrorhiza kurroa, Solanum indicum, Boerhavia diffusa, Andrographis paniculata, Cichorium intybus, Tephrosia purpurea, Eclipta alba.* Siddha and Ayurvedic pharmacopeia reports that these plants are having hepatoprotective activity. From literature review it was found that all these plants possess antioxidant and hepatoprotective activity individually but there is no scientific evident about their poly herbal combination. Thus, a proper scientific assessment has become the criteria for acceptance of herbal health claims. Hence the present study aims to investigate the hepatoprotective activity of Yakrit plihantak churna (YPC).

MATERIALS AND METHODS

Chemicals and drugs

Carbon tetrachloride and other chemicals used were of analytical grade and purchased from Lab chemicals, Chennai, Tamil Nadu, India. Silymarin was purchased from micro labs, Bangalore, Karnataka, India. Kits for biochemical parameters such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin, total protein and albumin were purchased from Accurex diagnostics, Mumbai, India.

Yakrit Plihantak Churna

Yakrit plihantak churna is a poly herbal formulation procured from Planet Ayurveda pvt Ltd, Mohali, India.

Experimental animals

Healthy wistar albino rats weighing between 120- 170 g were used for the study. The animals were procured from Animal experimental laboratory Madras medical college, Chennai, India. The animals were acclimatized to the laboratory conditions and they were housed in polypropylene cages, maintained on a 12-h light/ dark cycle and the animals were provided with standard pellet and free access to water. The study protocol was approved by the Institutional Animal Ethics Committee at Madras medical college, Chennai in accordance with the CPCSE A guidelines (PROPOSALNO:1917/ReBi/S/16/CPCSEA/25.06.2016) and all experiments were carried humanely.

Table 1: Yakrit Plihantak Poly herbal formulation

Plants used in the formulation	Part used	Amount
Phyllanthus niruri	Whole	60 gm
Picrorhiza kurroa	Whole	20 gm
Solanum nigrum	Whole	20 gm
Boerrhavia diffusa	Roots	20 gm
Andrographis paniculata	Roots	20 gm
Cichorium intybus	Stem	20 gm
Tephrosia purpurea	Whole	20 gm
Eclipta alba	Roots	20 gm

Acute oral toxicity

The animals had free access to water and food throughout the experiment, except for the fasting period before the oral administration of the single dose of the Yakrit plihantak churna. The YPC was administered as it is by gavages (orally) at single dose of 2000 mg/kg. The general behaviour and mortality of the rats was continuously monitored for 1 h after dosing periodically during first 24 h (with special attention given during the first 4 h) and then daily for a total of 14 days. Changes in the normal activity of rats, sign and symptoms of toxicity and mortality were monitored and recorded. Acute toxicity study was carried out as per OECD Guidelines 423.

Experimental design

The animals were divided into five groups (n = 6) where Group I served as the normal control group and received the normal feed and water for 35 days. CCl_4 (1 ml/kg body weight) was administered to animals of all the remaining groups on the 1st and 4th day of every week until the 35th day of the experiment by intraperitoneal injection with equal amount of olive oil. Group II was a CCl_4 control group and received only CCl_4: olive oil (1:1). Group III served as standard group and received silymarin (25 mg/kg body weight) which was administered orally with intra-gastric cannula for 35 days. Group IV and Group V received Yakrit plihantak churna, 200 and 400 mg/kg body weight was suspended in 1% w/v CMC and administered orally with intra-gastric cannula for 35 days. On the 36th day, blood samples were collected by cardiac puncture for estimation of biochemical parameters. The animals were sacrificed by high doses of inhalational anaesthesia. The liver was removed rinsed in ice cold saline and stored in 10% formalin solution. A part of liver was homogenised with Phosphate buffer, pH 7.4 using a Teflon homogenizer in ice-cold condition. The homogenate was centrifuged at 9000 rpm for 20 min. The supernatants solution was taken up for the evaluation of Lipid peroxidation (LPO) Superoxide dismutase (SOD) levels. The other part of liver was subjected to histopathological study.

Estimation of biochemical parameters

The blood collected in clot stimulating tubes was kept in room temperature for 30 min to separate the serum. The serum was transferred to fresh tubes and stored at -20°C until use. The serum samples were used to determine enzyme as well as other biochemical markers of liver function including aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin, total protein and levels of albumin. All the assays were carried out using standard diagnostic kits (Accurex Diagnostics Limited kits. Mumbai, India).

Estimation of oxidative stress markers

The oxidative stress markers were measured in liver tissue by using liver homogenate which was prepared by homogenizing the liver in 10% (w/v) ice cold buffer (0.1 M phosphate buffer, pH 7.4 + 150 mM potassium chloride). The crude homogenate was centrifuged at 9000 rpm for 20 min to obtain supernatant fraction. The supernatant fraction was then used for estimation of malondialdehyde (MDA) which is the marker of lipid peroxidation and Superoxide dismutase (SOD) levels.[10,11]

Histopathological studies

The liver from the animals were rinsed in 0.9% ice cold saline and fixed in 10% formalin embedded in paraffin and cut into 5 µm thick section using a microtome. Sections were mounted on glass slides using standard techniques. The sections are stained with Haematoxylin - Eosin and examined under a microscope using 10x magnifications and photographed under a light microscope.

Statistical analysis

All the values are expressed as mean ± SEM. The data was statistically analysed by one-way ANOVA followed by Dunnett's test using Graph pad prism version 8.0.1 and $P < 0.05$ was considered to be significant.

RESULTS

Acute oral toxicity study – OECD 423

Acute toxicity studies demonstrate the adverse effects occurring within a short period of time (up to 14 days) after administration of single dose of a test substance or after multiple doses within 24 h of starting point. The treatment with YPC did not produced any lethal or toxic symptoms up to a dosage of 2000 mg/kg body weight. No mortality or morbidity occurred in animals during 14-day period following an oral administration of YPC. Skin and fur color, salivation, lacrimation was found to be normal in all the

animals whereas aggressiveness, catatonia, writhing effect, diarrhoea, tremors were absent.

Body weight changes in animals

The body weight of animals was determined on 1st and 35th day of the study period in the CCl_4 treated group there was a considerable reduction in body weight on the 35th day as compared to the 1st day this shows that animals were affected by CCl_4 toxicity. Whereas the Silymarin and YPC treated group shows a weight gain when compared to 1st day this shows that YPC reduced the effects of CCl_4 exposure in animals. Results were given in Table 2 and Figure 1.

Effect of YPC on liver biochemical parameters

The extent of hepatic damage caused by the administration of CCl_4 was assessed by the quantitative estimation of both enzymatic and non-enzymatic hepatic function markers. Activity levels of serum AST, ALT, ALP and LDH were significantly ($p < 0.001$) elevated in CCl_4 treated animals when compared to the normal group. Results were reported in Table 2 and Figure 2-5. Total serum protein, albumin was significantly ($p < 0.001$) reduced in CCl_4 treated animals, while bilirubin showed significant ($p < 0.001$) elevation when compared to the normal control. Results were given in Table 3 and Figure 6-8. Post treatment with YPC significantly improved the liver condition that reflected in the biochemical parameters as they tend to shift towards the normal values. The elevated levels of bilirubin were significantly ($p < 0.001$) reduced whereas the depleted levels of total protein and albumin improved significantly ($p < 0.001$) upon treatment with YPC. The change of biochemical parameters after YPC administration was highly significant ($p < 0.001$) in a dose dependent manner. The ameliorative effects of YPC at the dose of 400 mg/kg body weight were found comparable with standard drug silymarin.

Effect of YPC on oxidative stress markers

The status of hepatic antioxidant as well as lipid peroxidation level was drastically affected with CCl_4 administration as evident from the altered levels of biochemical markers such as SOD and MDA. A significant ($p < 0.001$) increment in the level of hepatic tissue MDA was recorded in CCl_4 treated animals when compared to normal control. The activity levels of liver antioxidant defense enzyme SOD was significantly ($p < 0.001$) decreased in CCl_4 treated animals. Treatment with YPC showed a significant ($p < 0.001$) reduction in MDA level along with a significant ($p < 0.001$) elevation in the activity levels of SOD in liver. Results were given in the Table 4 and Figure 9 and 10.

Histopathological examination

Histopathology is the most important technique used to study the cytological changes occurred in the treatment groups. The livers were collected on 36th day and they were stained with Hematoxylin and Eosin and sectioned for cytological examination. The results are shown in Figure 11.

Liver sections of normal control Figure 11(a) showed the normal liver architecture. Hepatocytes, portal tract are normal in size and Shape. The central vein and sinusoids are normal. In Disease control group Figure 11(b and c) the liver parenchyma with the hepatocytes showing steototic change, centrilobular necrosis, periportal inflammatory infiltration composed of lymphocytes, minimal fibrosis and congested and dilated sinusoids was observed. The histological profile of Silymarin treated group Figure 11(d) Showed almost normal hepatic architecture with mild dilation of sinusoids. In the group treated with YPC (200 mg/kg) Figure 11(e) the section showed liver parenchyma with mild periportal inflammation along with lymphocyte infiltration and sinusoidal dilation and in YPC (400 mg/kg) treated group Figure 11(f) showed almost normal hepatic architecture with minimal sinusoidal dilation along with reduced portal triad inflammation.

Table 2: Effect of YPC on body weight of the treated animals and liver biochemical parameters

Groups	Group names	Animal body weight in grams		AST IU/L	ALT IU/L	ALP IU/L	LDH IU/L
		Initial	Final				
I	Normal control	150 ± 1.89	167 ± 1.72	124.6 ± 2.40	48.8 ± 1.37	233.8 ± 2.53	2095 ± 2.20
II	Disease control	157.5 ± 1.92	133.3 ± 3.52	290 ± 1.89$^{###}$	157.8 ± 2.71$^{###}$	440 ± 1.72$^{###}$	2434 ± 10.27$^{###}$
III	Silymarin	133.8 ± 1.51	136 ± 2.94	139.3 ± 1.89***	56.1 ± 2.89***	254.6 ± 2.82***	2184 ± 1.95***
IV	YPC (200 mg/kg)	144 ± 2.70	145.8 ± 3.00	164 ± 7.38***	94.6 ± 1.08***	336.3 ± 3.06***	2360 ± 3.46***
V	YPC (400 mg/kg)	148.3 ± 5.42	152.3 ± 4.99	134 ± 3.30***	53.1 ± 1.22***	259 ± 2.90***	2192 ± 1.6***

All the values are expressed as mean ± SEM (n = 6),
$^{###}P < 0.001$ compared to normal control group, $^{***}P < 0.001$ compared to disease control group

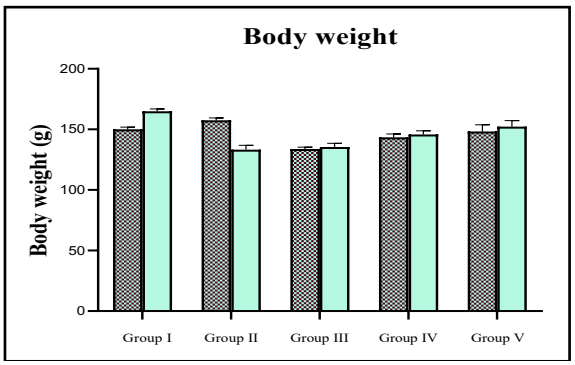

Figure 1: Effect of YPC on body weight of treated animals

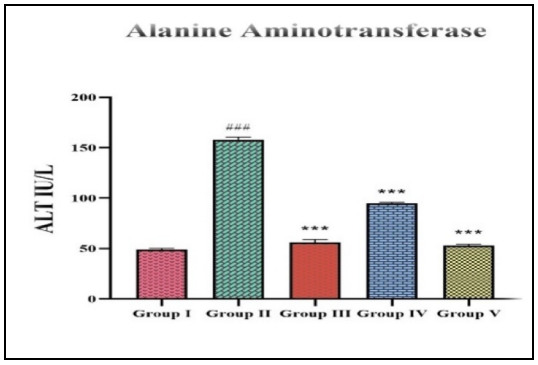

Figure 2: Effect of YPC on ALT levels

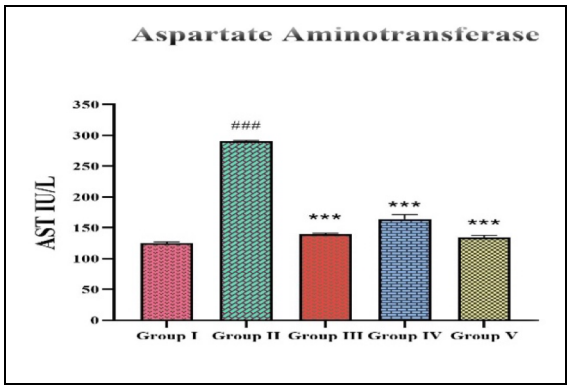

Figure 3: Effect of YPC on ALT levels

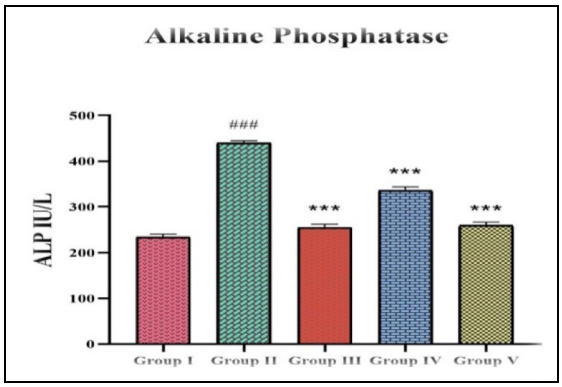

Figure 4: Effect of YPC on ALP levels

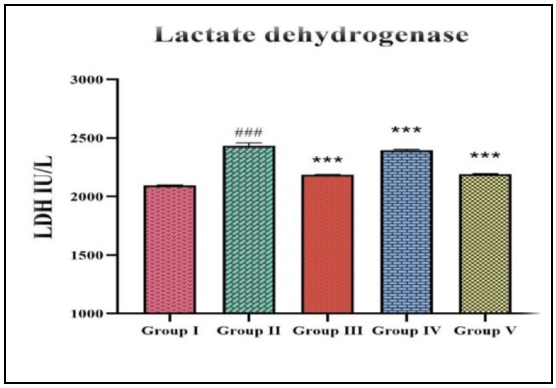

Figure 5: Effect of YPC on LDH levels

Table 3: Effect of YPC on non-enzymatic biomarkers of liver

Groups	Group names	Total Bilirubin mg/dL	Total protein mg/dL	Albumin gms/dL
I	Normal control	0.117 ± 0.02	7.7 ± 0.05	2.6 ± 0.05
II	Disease control	0.303 ± 0.02$^{###}$	6.6 ± 0.05$^{###}$	2.08 ± 0.08$^{###}$
III	Silymarin	0.133 ± 0.01***	7.31 ± 0.04***	2.51 ± 0.03***
IV	YPC (200 mg/kg)	0.167 ± 0.01***	7.08 ± 0.05***	2.31 ± 0.03*
V	YPC (400 mg/kg)	0.143 ± 0.01***	7.45 ± 0.03***	2.48 ± 0.03***

All the values are expressed as mean ± SEM (n = 6),
$^{###}P < 0.001$ compared to normal control group, $^{***}P < 0.001$ compared to disease control group, $^{*}P < 0.05$ compared to disease control group

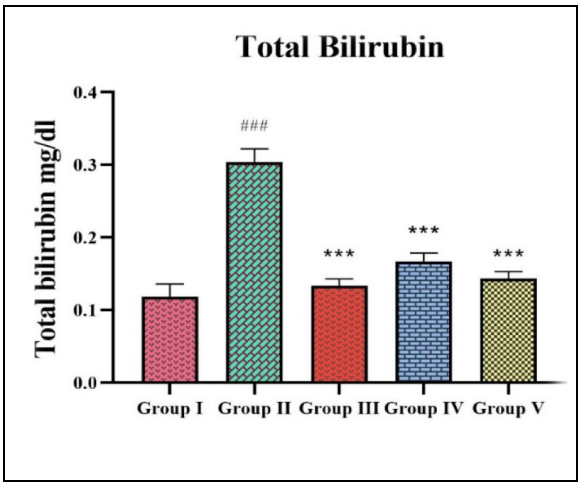

Figure 6: effect of YPC on Total bilirubin

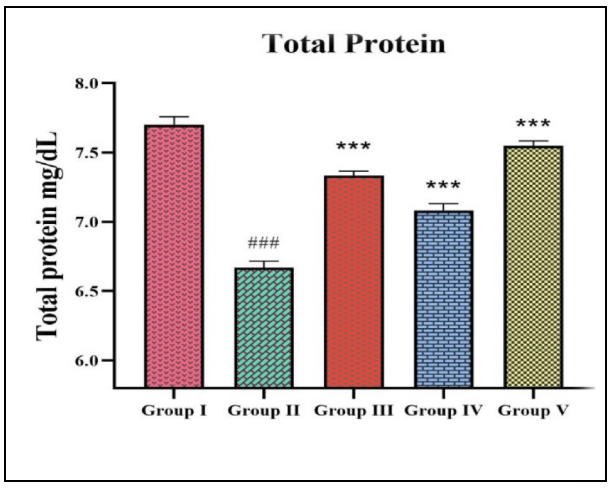

Figure 7: Effect of YPC on Total protein

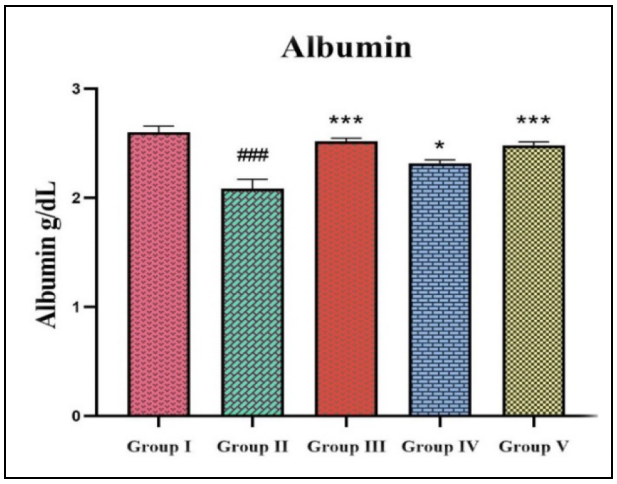

Figure 8: Effect of YPC on Albumin levels

Table 4: Effect of YPC on oxidative stress markers of liver

Groups	Group names	MDA nmoles/gram of tissue	SOD U/ g of tissue
I	Normal control	4.5 ± 0.10	7.2 ± 0.18
II	Disease control	8.6 ± 0.34$^{###}$	3.8 ± 0.16$^{###}$
III	Silymarin	5.1 ± 0.13***	6.6 ± 0.15***
IV	YPC (200 mg/kg)	6.7 ± 0.11***	5.6 ± 0.1***
V	YPC (400 mg/kg)	5.4 ± 0.33***	6.5 ± 0.17***

All the values are expressed as mean ± SEM (n = 6),
$^{###}P < 0.001$ compared to normal control group, $^{***}P < 0.001$ compared to disease control group

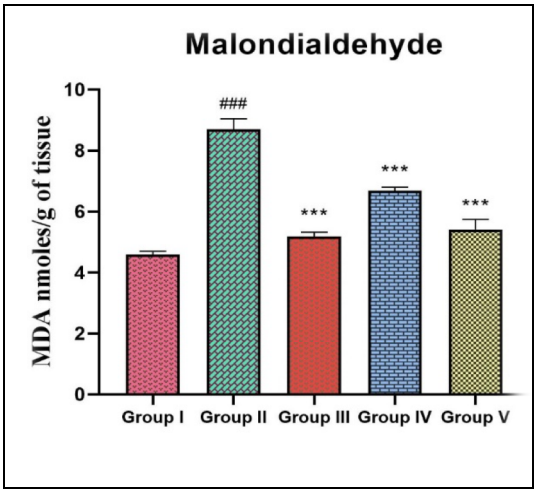

Figure 9: Effect of YPC on MDA levels

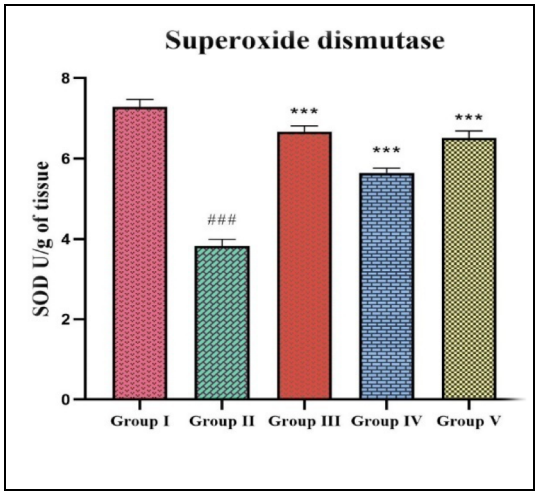

Figure 10: Effect of YPC on SOD levels

Histopathological examination

GROUP I

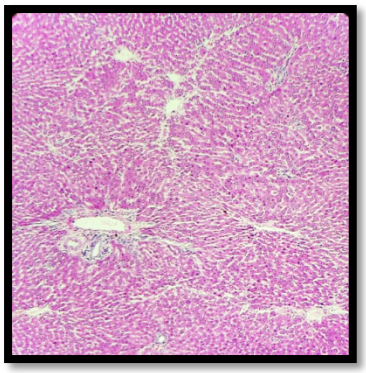

Figure 11(a): Normal control

GROUP II

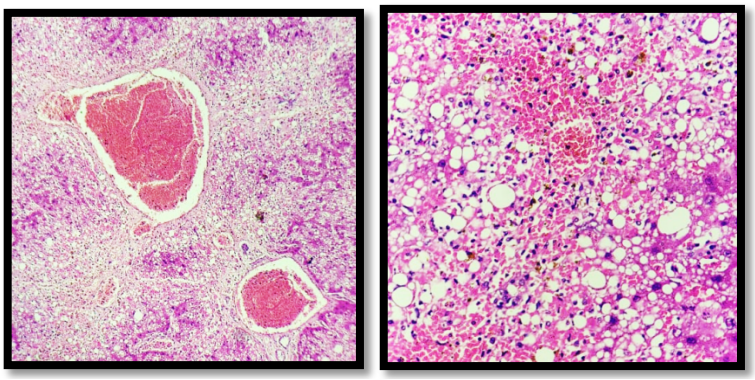

Figure 11(b) and (c): Disease control

GROUP III **GROUP IV**

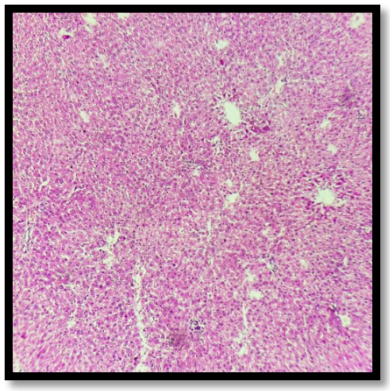

Figure 11(d): CCl_4 + Silymarin treated group

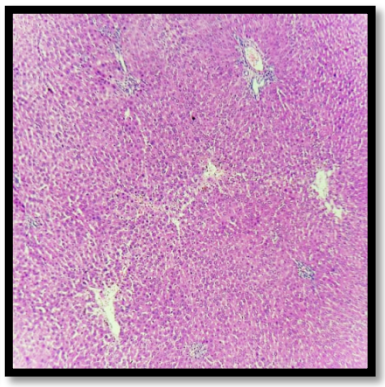

Figure 11(e): CCl_4 + YPC (200 mg/kg) treated group

GROUP V

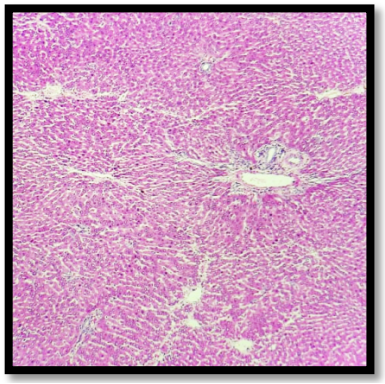

Figure 11(f): CCl_4 + YPC (400 mg/kg) treated group

DISCUSSION

Liver disease has been a major health problem globally. Liver disease accounts for approximately 2 million deaths per year worldwide, 1 million dues to complications of cirrhosis and 1 million due to viral hepatitis and hepatocellular carcinoma (HCC). Globally, cirrhosis currently causes 1.16 million deaths, and liver cancer 788,000 deaths each year.[12] Around 10 lakhs patients of liver cirrhosis are newly diagnosed every year in India. Liver disease is the tenth most common cause of death in India as per the WHO Liver cirrhosis is the 14th leading cause of deaths in the world and could be the 12th leading cause of deaths in world by 2020.

Hepatocellular carcinoma (HCC), or a cancer in liver, is the second most common cause of death due to malignancy in the world.[13] This report gives an alarm, to decrease the morbidity and mortality rate caused by hepatic complications; one of the most common problems in the world is hepatic diseases. If it is persisting for long time the morbidity and mortality will be increased. So we have to make use the most of the existing formulations or to evaluate new treatments. Plants have provided mankind with herbal remedies for many diseases. Healing with medicinal plants is as old as mankind itself. The connection between man and his search for drugs in nature dates from the far past. Knowledge of medicinal plants usage is an effect of the many years of struggles against illnesses due to which man learned to pursue drugs in barks, seeds, fruit bodies, and other parts of the plants.[14]

The Indian system of medicine, especially Ayurveda, has several medicinal plants with proven beneficial claims towards many pathological conditions. However, the potential of herbal drugs as defined therapeutic agents is undetermined by the difficulty in standardization, pharmacodynamics and pharmacokinetics of these multi component mixtures and also the lack of enough experimental data. Medicinal plants need to be investigated scientifically and rigorously to define their role in prevention and treatment of pathological conditions and to stimulate the future pharmaceutical development of therapeutically beneficial herbal drugs. Many herbals are on market to support health, relieve symptoms and cure diseases. However, most of these products lack scientific evidence that supports these allegations. One such product is Yakrit plihantak churna. Since; No pharmacological studies have been done on this poly herbal formulation.

Taking this consideration in our mind, we have planned this study for evaluation of the protective effect of poly herbal formulation especially with a definite aim of hepatoprotection. The present study was aimed to evaluate the hepatoprotective effect of the Yakrit plihantak churna against CCl_4 induced toxicity in rats.

CCl_4 is a well-known hepatotoxin that is widely used to induce hepatotoxicity in laboratory animals. CCl_4 induced hepatotoxicity in rat's represents an adequate experimental model of fibrosis/cirrhosis in human and it is used for the screening of hepatoprotective drugs. Carbon tetrachloride- (CCl_4) provoked hepatotoxicity has been widely explored in hepatology. Covalent binding of the CCl_3^* radical to cell components prevents lipoprotein secretion and thus initiates steatosis, whereas reaction with oxygen to form CCl_3-OO* introduces lipid peroxidation which in consequence results in apoptosis and cell death.[7]

Safety is a fundamental principle in the provision of herbal products for health care. To assess the safety and toxicity manifestations acute oral toxicity study was carried out in wistar rats. Oral administration of YPC at a dose of 2000 mg/kg did not produce any mortality or significant changes in their behaviour.

Measurement of the activity levels of enzymes in the body fluids is a useful monitor of disease state. An obvious sign of hepatic injury is leakage of cellular enzymes into serum.[15,16] Serum ALT activity is the most frequently used biomarker. AST, often used along with ALT is another biomarker employed to assess liver function. These two biomarkers are involved in the reductive transfer of an amino group from alanine or aspartate, respectively, to alpha-ketoglutarate to yield glutamate and pyruvate or oxaloacetate, respectively. The damaged hepatocytes release ALT and AST into the extracellular space and ultimately enter the blood stream thereby increase the serum levels of these biomarkers. Alkaline phosphatase is considered as cholestatic induction markers of hepatobiliary origin that can be linked to impaired bile flow. ALP is an extracellular hydrolytic enzyme which is eliminated in the bile. In addition to liver it is also found in placenta, bone, kidney and intestine. An increase in ALP levels is suggestive of liver or bile duct diseases. In the present study, the elevated levels of ALP were increased in the rats treated with CCl_4 suggested the hepatic injury. Serum level of LDH is another enzyme biomarker of hepatocellular toxicity but with less specificity.

In the present study, the elevated levels of serum AST, ALT, ALP and LDH in CCl_4 treated animals might be due to damage of liver tissue and/or changes in cell permeability that allowed these enzymes to leak into serum. Treatment with YPC significantly reduced the biomarker levels. This suggests that YPC was able to repair the probable hepatic injury and/or restore the cellular

permeability; thus reducing the toxic effect of CCl_4 in the liver tissue.

Bilirubin is the conventional indicator of liver diseases. Hyperbilirubinemia is a very Sensitive test to substantiate the functional integrity of the liver and severity of necrosis which increases the binding, conjugating and excretory capacity of hepatocytes that is proportional to the erythrocyte degeneration rate. The concentration of bilirubin was significantly increased in CCl_4 toxicity induced group. Depletion of the elevated bilirubin level in the serum of rats treated with YPC, suggests that YPC have improved the liver metabolism.

Protein synthesis is a function of the healthy liver and thus total protein levels becomes a distinguishing factor of normal and damaged liver. Hypoproteinaemia occurs due to impaired synthesis function of liver as a result of hepatotoxicity. Albumin is a major protein that circulates within the blood stream and low serum albumin levels is an indicator of hepatotoxicity.

Diminution of Total protein and Albumin induced by CCl_4 is a further indication of liver damage. YPC has increased the levels of serum TP and ALB towards the respective normal value, which indicates hepatoprotective activity. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism which accelerates the regeneration process and the production of liver cells.

During CCl_4 induced liver toxicity there will be excessive generation of free radicals. Free radicals are the ROS and are known to cause oxidative damage to a number of molecules in the cell, including membranes-lipids, proteins and nucleic acids. In the present study, the hepatic cellular injury might be due to increased oxidative stress that led to lipid peroxidation.

The levels of lipid peroxidation in the CCl_4 treated rats were assessed by measuring the MDA levels in the liver tissue. The increased MDA levels in the liver of CCl_4 treated animals indicate enhanced lipid peroxidation leading to tissue injury. Treatment with YPC shown a significant depletion of levels of MDA in the liver tissue which suggest that YPC might reduce the lipid peroxidation and elevated the antioxidant defense enzyme activity.

The cellular antioxidant defense mechanisms, which include scavenging activities of enzyme like SOD play an important role in scavenging toxic intermediates of ROS generation. During hepatotoxicity these enzymes are structurally and functionally impaired by free radicals resulting in liver damage. Numerous studies have shown the importance of SOD in protecting cells against oxidative stress. The decreased activity of SOD in the liver tissue of CCl_4 treated animals could be due to the feedback inhibition or oxidative inactivation of enzyme protein due to excess ROS generation. Treatment with YPC shows a significant increment in the activity levels of SOD indicating the free radical scavenging activity of YPC. Similarly, reduction in lipid peroxidation tends to increase the antioxidant enzyme activity levels during YPC supplementation.

The biochemical findings were supported with histopathological observations of liver sections. The normal control group showed normal cellular architecture with sinusoidal spaces and central veins while CCl_4 treated group revealing centrilobular hepatic necrosis and showed mild vacuolated cells that suggested the fatty degeneration. In YPC treated groups hepatocellular changes were restored towards normalcy. These observations suggest that this formulation was able to control this necrotic change that was comparable to that of silymarin.

CONCLUSION

From these results we concluded that the Yakrit plihantak churna was efficiently combating the CCl_4 induced oxidative stress and liver injury. The biochemical markers of liver injury and oxidative stress markers in addition to the histopathological evaluation clearly revealed the restorative effect of YPC against liver injury and the hepatoprotective activity of YPC may be due to the fact that they contain a combination of different phytochemicals, such diversity of these compounds magnified their potential, so that it can be recommended for pharmaceutical importance.

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