Research paper

AMELIORATIVE EFFECT OF LEAVES OF *CARICA PAPAYA* IN ETHANOL AND ANTITUBERCULAR DRUG INDUCED HEPATOTOXICITY

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ABSTRACT

Aims: The leaves of *Carica papaya* Linn. (Caricaceae) have been traditionally used as a medicine against some forms of liver diseases. Its hepatoprotective activity against hepatotoxicants like CCl_4 and paracetamol has been scientifically proved in animals. The aim of the present study is to examine the hepatoprotective effect of *C. papaya* leaves against ethanol and anti-tubercular drug-induced liver damage, the two clinically relevant animal models.

Study Design: The aqueous extract of leaves of the plant was administered orally to rats and effects on hepatic marker enzymes, tissue antioxidants and liver histology were determined.

Place and Duration of Study: The study was performed in Rayat Institute of Pharmacy, Railmajra, Dist. S.B.S. Nagar, Punjab, between Dec' 2011 to July 2012.

Materials and Methods: The study was performed by administering the aqueous extract of *C. papaya* leaves at the dose of 400 mg/kg in rats prior to administration of ethanol or combination of Isoniazid and Rifampicin. The effects on the levels of serum indicators of liver damage (SGPT, SGOT, alkaline phosphatase and total bilirubin) and tissue antioxidant parameters like TBARS, GSH and SOD were determined. Histopathology of liver was also performed to study the influence of drug on tissue integrity.

Results: Hepatoprotective activity of leaves of *C. papaya* was evident by the significant reduction in the levels of all serum markers in both the models. The extract also significantly increased the levels of SOD, GSH and total protein; and decreased the levels of TBARS, indicating its antioxidant effect. The results obtained were also confirmed by improvements in the histopathology of liver.

Conclusion: The results obtained in the current study justify the use of *Carica papaya* leaves in the treatment of liver damage induced by commonly consumed hepatotoxicants, namely ethanol and anti-tubercular drugs.

Keywords: *Carica papaya; leaves; ethanol; hepatotoxicity; antioxidant; histopathology.*

1. INTRODUCTION

Liver disease is the major cause of death worldwide. It ranks ninth in overall causes of death in the U.S. [1] and is the fifth 'big killer' in England & Wales. In India too, liver diseases are responsible for lakhs of deaths each year. It is rather the only major cause of death still increasing year-on-year.

Alcoholic liver disease (ALD) is among the leading causes of morbidity and mortality in the world. In an assessment by the WHO in 2005, 4% of the burden of the disease and 3.2% of all deaths globally were attributable to alcohol. The burden of alcohol-related disease is highest in the developed world, where it may account for as much as 9.2% of all disability-adjusted life years [2]. Even in the developing regions of the world, however, alcohol accounts for a major portion of global disease burden, and is projected to take on increasing importance in those regions over time [3].

Tuberculosis is one of the fatal communicative diseases and is spread easily amongst people. Over one-third of the world's population is estimated to be infected with *Mycobacterium tuberculosis* and over 2 million people a year die of the disease. The first line anti-tubercular drugs namely, Rifampicin and Isoniazid are potentially hepatotoxic drugs. Based on hepatotoxicity diagnosis criteria and population under study, incidence of anti-TB related hepatotoxicity is reported to range from 2% to 28% [4].

The available therapeutic interventions for liver diseases, which include drugs like steroids, anti-cytokines, colchicine or supplementation with calorie rich diet or a precursor of glutathione, S-adenosyl L-methionine (SAMe) have not been able to show convincing benefit in humans and also suffer from several side effects. Thus herbal medicines have an important role to play in the treatment of liver disorders considering their efficacy, safety and lesser side effects.

A wide range of plants have been utilized for the treatment of multiple disorders of the liver. There are hundreds of hepatoprotective herbs which are proven for their hepatoprotective action against several types of hepatotoxins. A few important examples are *Silybum marianum, Phyllanthus niruri, Andrographis paniculata, Picrrorhiza kurroa, Eclipta alba* and *Tephrosia purpurea.*

Carica papaya Linn. is a tropical, herbaceous plant, belonging to family Caricaceae. Traditionally young leaves have been used in jaundice (as fine paste), urinary complaints, gonorrhoea (as infusion), colic, fever, beriberi, abortion (as infusion) and in asthma (as smoke). The leaves of *Carica papaya* have been recently studied against hepatotoxins like CCl_4 [5] and paracetamol [6] and have indicated their hepatoprotective activity in rats. The leaves of *Carica papaya* have however not been proved for its efficacy against clinically important and widely ingested hepatotoxicants, namely ethanol and antitubercular drugs. Thus, the purpose of the present study was to evaluate the hepatoprotective activity of the aqueous extract of leaves of *Carica papaya* against ethanol and anti-tubercular drug- induced liver toxicity.

2. MATERIAL AND METHODS

2.1 Drugs and Chemicals

Silymarin tablets (Silybon-70®, Micro Labs Ltd., Himachal Pradesh, India) were purchased from the local market. All the reagents and chemicals used in the study were of analytical grade and were procured from Spruce Enterprises (Ambala, Haryana, India). Diagnostic kits for the estimation of serum levels of various parameters were procured from Avecon Healthcare Pvt. Ltd. (Saha, Haryana, India).

2.2 Collection of Plant Material

Fresh leaves of *Carica papaya* Linn. (Family: Caricaceae) were collected from the botanical garden of Rayat Institute of Pharmacy, Railmajra (Punjab). The leaves of *C. papaya* were identified and authenticated by Dr. S.K. Upadhyay, Department of Horticulture, CSK Himachal Pradesh Agriculture University, Palampur, Himachal Pradesh (India).

2.3 Preparation of Extract

The leaves were washed with water to eliminate any dead matter and other unwanted particles and then air-dried for 3 weeks. The dried leaves were then ground into a coarse powder, which was then boiled in distilled water for an hour, and subsequently filtered through Whatman filter paper (No.1). The filtered solution was then heated at 60^{o}–70^{o}C to yield a concentrated solution, which was then vacuum dried. The

percentage yield was found to be 10.5%. The aqueous extract of *C. papaya* leaves (AECPL) was dissolved in distilled water and prepared fresh for the experiments.

2.4 Phytochemical evaluation

Phytochemical evaluation of aqueous extract of leaves of *Carica papaya* (AECPL) was carried out as per standard methods [7, 8].

2.5 Experimental animals

Albino Wistar rats of either sex (weighing 150-180 g) were used for the experiments. The animals were housed in polypropylene cages under standard conditions (Temperature: 28 ± 2ºC, Relative humidity: 50 ± 2%, 12 hr light / dark cycle) and provided with standard pellet diet and water *ad libitum.* The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

2.6 Grouping of Animals

Animals were divided into four groups of six animals each. Group 1 represented the normal control group that received the vehicle, distilled water (5ml/kg, p.o.). Group 2 represented the negative control group which received the vehicle, distilled water (5ml/kg, p.o.) followed by administration of the hepatotoxicant. Group 3 represented the drug treated group which received the aqueous extract of leaves of *Carica papaya* (AECPL) at the dose of 400 mg/kg (p.o.). Group 4 served as the standard drug treated group, which received silymarin at the dose of 200 mg/kg (p.o.).

2.7 Experimental Methodology

2.7.1 Ethanol induced hepatotoxicity

The method of Zhang et al. [9] was adopted with slight modification. The rats were fasted overnight. All the animals received the treatment (single dose) as mentioned above, which was followed 1 hr later by administration of absolute ethanol (4.5 ml/kg, p.o.) to animals of Groups 2, 3 and 4. After 18 hrs of ethanol

administration, blood was collected by the retro-orbital plexus method and serum was separated for the estimations of SGPT, SGOT, ALP and total bilirubin. The animals were then sacrificed and the liver was dissected out, weighed and homogenized (Homogenizer REMI RQM-122, Remi Instrument, India) in chilled phosphate buffer (50 mM, pH 7.4) to give a concentration of 10% w/v. The resultant homogenate was then centrifuged at 4°C and the clear supernatant was collected. This supernatant was then used for assays of TBARS (Thiobarbituric acid reactive substance), superoxide dismutase (SOD), reduced glutathione (GSH) and proteins. Histopathological studies on liver were also carried out.

2.7.2 Antitubercular drugs- induced hepatotoxicity

The method of Jiang *et al.* [10] and Saleem *et al.* [11] was adopted. Briefly, the animals were pretreated for a period of 10 days. During the 10 days of drug treatment the animals of Groups 2, 3 and 4 received the combination of anti-tubercular drugs (Isoniazid and Rifampicin) at the dose of 125 mg/100g (i.p.) 1 h after the administration of the drugs. On the 10^{th} day, one hour after administration of the last dose of the anti-tubercular drugs, blood was collected and serum was separated for determining the levels of SGPT, SGOT, ALP and total bilirubin. The liver was also removed and processed as mentioned above. The clear supernatant was then used for assays of TBARS (Thiobarbituric acid reactive substances), reduced glutathione (GSH) and proteins. Histopathological studies on liver were also carried out.

2.8 Tissue Estimations

The quantitative measurement of thiobarbituric acid reactive substances (TBARS), which is an index of extent of lipid peroxidation, was performed according to the method of Ohkawa et al. [12]. The levels of reduced glutathione (GSH) and Superoxide dismutase (SOD) were determined as per the methods of Beutler et al. [13] and Misra and Fridovich [14], respectively. The total protein content in the supernatant was determined by Lowry's method [15].

2.9 Histological studies

The liver was excised quickly and fixed in 10% buffered-formaldehyde at room temperature. After dehydration using graded ethanol, pieces of tissues were embedded in paraffin, cut into fine (5 µm)

sections and mounted on glass slides. Sections were then deparaffinized with xylene, counterstained with hematoxylin and eosin and viewed under a light microscope at 200 X.

2.10 Statistical Evaluation

The results were expressed as Mean ± SEM. Statistical analysis was carried out by using One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, with the level of significance set at $P < 0.05$.

3. RESULTS

3.1 Preliminary Phytochemical Analysis of AECPL

The results of the preliminary phytochemical screening of AECPL have been presented in Table 1. AECPL showed the presence of alkaloids, flavonoids, reducing sugar, saponins and tannins.

Table 1. Phytochemical Screening of aqueous extract of *Carica papaya* leaves (AECPL)

Sr. No.	TEST	PRESENT (+) / ABSENT (-)
1.	Alkaloids	+
2.	Flavonoids	+
3.	Reducing Sugar	+
5.	Saponins	+
6.	Tannins	+
7.	Glycosides	-
8.	Steroids	-
9.	Triterpenoids	-

3.2 Effects of AECPL in Ethanol-Induced Hepatotoxicity Model

3.2.1. Serum Parameters

Ethanol administration in the negative control group (Group 2) animals resulted in hepatic damage as evidenced by a significant ($P < 0.001$) increase in the levels of SGPT, SGOT, ALP and total bilirubin as compared to the normal control group (Group 1). Administration of AECPL to animals of Group 3 showed a significant ($P < 0.001$) reduction in the levels of SGPT, SGOT, ALP and total bilirubin when compared to the negative control group (Group 2). The effect produced by administration of 400 mg/kg of AECPL was found to be similar ($P > 0.05$) to that produced by 200 mg/kg of Silymarin (Table 2).

Table 2. Effect of aqueous extract of *Carica papaya* leaves (AECPL) on Serum Parameters in Ethanol Induced Hepatotoxicity Model in Rats

GROUPS	SGPT (U/L)	SGOT (U/L)	ALP (mg/dl)	TOTAL BILIRUBIN (mg/dl)
Group 1	37.51 ± 0.55	31.40 ± 0.50	28.82 ± 0.22	0.39 ± 0.03
Group 2	311.80 ± 26.02$^{###}$	201.00 ± 12.32$^{###}$	117.20 ± 1.87$^{###}$	1.33 ± 0.05$^{###}$
Group 3	69.82 ± 0.56***	88.29 ± 1.33***	39.05 ± 0.06***	0.68±0.03***
Group 4	57.21 ± 2.25***	69.00 ± 7.17***	33.79 ±1.59***	0.54 ± 0.05***

All values are expressed as Mean ± SEM. ###$P < .001$ when compared with Normal Control group (Group 1), ***$P < .001$ when compared with Negative Control group (Group 2)

3.2.2. Tissue Parameters

As regards to the tissue parameters, ethanol administration in negative control group (Group 2) animals showed a significant ($P < 0.001$) decrease in the levels of SOD, GSH and total protein; whereas the level of TBARS was found to be significantly ($P < 0.001$) increased as compared to normal control group (Group 1). Treatment of AECPL to the Group 3 animals showed a significant rise in the levels of SOD ($P < 0.01$), GSH ($P < 0.001$) and total protein ($P < 0.001$); whereas the level of TBARS was found to be

192 significantly (P < 0.001) lowered as compared to the negative control group (Group 2). Silymarin
193 administration in the Group 4 animals showed a significant (P < 0.001) increase in the levels of SOD, GSH
194 and total protein; whereas the level of TBARS was found to be significantly (P < 0.001) reduced as
195 compared to negative control group (Group 2). The effects produced on levels of TBARS and GSH by
196 administration of 400 mg/kg of AECPL (Group 3) were found to be similar to that produced by Silymarin
197 (Table 3).
198
199 **Table 3. Effect of Aqueous extract of *Carica papaya* leaves (AECPL) on Tissue Parameters**
200 **in Ethanol Induced Hepatotoxicity Model in Rats**

GROUPS	TBARS (nmoles/mg protein)	SOD (Units/mg protein)	GSH (µM/mg protein)	TOTAL PROTEIN (mg/g wet tissue)
Group 1	10.60 ± 0.50	4.35 ± 0.15	1.83 ± 0.05	2.75 ± 0.06
Group 2	43.33 ± 1.10$^{###}$	1.61 ± 0.14$^{###}$	0.74 ± 0.03$^{###}$	1.48 ± 0.06$^{###}$
Group 3	22.02 ± 0.19***	2.66 ± 0.19**	1.32 ± 0.01***	2.18 ± 0.03***
Group 4	16.75 ± 0.41***	3.72 ± 0.04***	1.74 ± 0.04***	2.45 ± 0.03***

201 All values are expressed as Mean ± SEM. ###P < .001 when compared with Normal Control group (Group 1), **P < .01; ***P < .001 when compared
202 with Negative Control group (Group 2)

203 **3.2.3. Histopathological Changes**

204 In the vehicle-treated normal control group (Group 1) animals, hepatocytes showed a normal lobular
205 architecture arranged in the form of cords and the presence of slight vascular congestion and a central
206 vein **[Fig.1.(A)]**; whereas in the negative control group (Group 2) animals, the hepatocytes showed
207 feathery degeneration and microcellular fatty changes, along with presence of inflammatory cells around
208 the portal tract and high vascular congestion **[Fig.1.(B)]**. In the liver of animals which were treated with
209 AECPL (Group 3) the hepatocytes showed minimal microcellular fatty changes and slight vascular
210 congestion **[Fig. 1.(C)]**. In the silymarin treated group (Group 4) the liver sections showed slight vascular

congestion and the hepatocytes were found to be arranged in the form of cords, similar to that in the normal control group **[Fig.1.(D)]**.

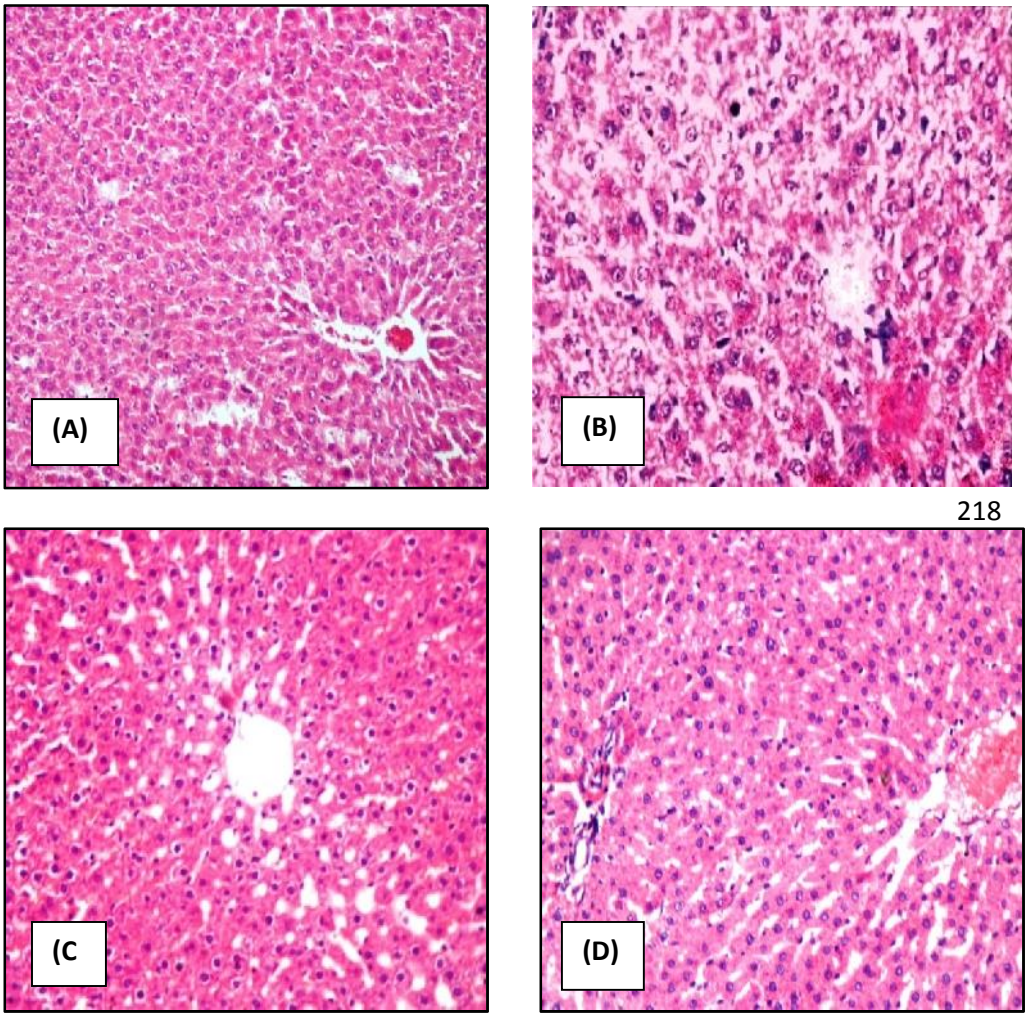

Fig. 1. Histopathological Studies of Liver in Ethanol Induced Hepatotoxicity

(A) Normal Control (Group 1); (B) Negative Control (Group 2); (C) AECPL + Ethanol (Group 3); (D) Silymarin + Ethanol (Group 4)

3.3. Effects of AECPL in Antitubercular Drugs Induced Hepatotoxicity Model

3.3.1 Serum Parameters

Administration of the anti-tubercular drug combination (INH+RIF) in the negative control group (Group 2) animals resulted in a significant ($P < 0.001$) increase in the levels of SGPT, SGOT, ALP and total bilirubin when compared to the normal control group (Group 1). Administration of AECPL to animals of Group 3

showed a significant (P < 0.001) reduction in the levels of SGPT, SGOT, ALP and total bilirubin as compared to the negative control group (Group 2). Similarly a significant (P < 0.001) reduction in the levels of all serum parameters was observed in the Silymarin treated Group 4 animals as compared to the negative control group (Group 2). The effect produced by administration of AECPL was found to be similar (Non-significant; P > 0.05) to that produced by 200 mg/kg of Silymarin (Table 4).

Table 4. Effect of aqueous extract of *Carica papaya* leaves (AECPL) on Serum Parameters in Anti-tubercular drugs Induced Hepatotoxicity Model in Rats

GROUPS	SGPT (U/L)	SGOT (U/L)	ALP (mg/dl)	TOTAL BILIRUBIN (mg/dl)
Group 1	37.51 ± 0.55	31.40 ± 0.50	28.82 ± 0.22	0.39 ± 0.03
Group 2	218.80 ± 14.70$^{###}$	308.40 ± 18.16$^{###}$	251.90 ± 10.78$^{###}$	1.71 ± 0.05$^{###}$
Group 3	139 ± 5.53***	175.3 ± 4.4***	150.5 ± 5.7***	0.82 ± 0.05***
Group 4	108.90 ± 4.19***	138.90 ± 4.61***	124.00 ± 4.2***	0.63 ± 0.03***

All values are expressed as Mean ± SEM. ###P < .001 when compared with Normal Control group (Group 1), ***P < .001 when compared with Negative Control group (Group 2)

3.3.2. Tissue Parameters

Antitubercular drugs (INH+RIF) administration in negative control group (Group 2) animals showed a significant (P < 0.001) decrease in the levels of SOD, GSH and total protein; whereas the level of TBARS was found to be significantly (P < 0.001) increased as compared to normal control group (Group 1). Treatment of AECPL to the Group 3 animals showed a significant rise in the levels of GSH (P < 0.01) and total protein (P < 0.01); whereas the level of TBARS was found to be significantly (P < 0.01) lowered as compared to the negative control group (Group 2). Silymarin administration in the Group 4 animals showed a significant (P < 0.001) increase in the levels of SOD, GSH and total protein; whereas the level of TBARS was found to be significantly (P < 0.001) reduced as compared to negative control group

(Group 2). The effects produced on levels of TBARS and GSH by administration of AECPL (Group 3) were found to be similar to that produced by Silymarin (Table 5).

Table 5. Effect of Aqueous extract of *Carica papaya* leaves (AECPL) on Tissue Parameters in Anti-tubercular drugs Induced Hepatotoxicity Model in Rats

GROUPS	TBARS (nmoles/mg protein)	GSH (µM/mg protein)	TOTAL PROTEIN (mg/g wet tissue)
Group 1	10.60 ± 0.51	1.83 ± 0.05	2.68 ± 0.06
Group 2	52.53 ± 1.08$^{###}$	0.54 ± 0.02$^{###}$	1.37 ± 0.04$^{###}$
Group 3	34.03 ± 0.7**	0.75 ± 0.02**	1.70 ± 0.01**
Group 4	22.14 ± 0.32***	1.48 ± 0.04***	2.01 ± 0.05***

All values are expressed as Mean ± SEM. ### P < .001 when compared with Normal Control group (Group 1); ** P < .01; *** P < .001 when compared with Negative Control group (Group 2)

3.3.3. Histopathological Changes

In the vehicle-treated normal control group (Group 1) animals, hepatocytes showed a normal lobular architecture arranged in the form of cords, presence of slight vascular congestion and a central vein **[Fig.2.(A)]**; whereas in the negative control group (Group 2) animals**,** hepatocytes showed feathery degeneration, microcellular fatty changes, portal triaditis and vascular congestion in the liver **[Fig.2.(B)]**. In the AECPL treated group (Group 3) the hepatocytes showed a minimal vascular congestion with moderate portal triaditis and microcellular fatty changes **[Fig. 2.(C)]**. In the silymarin treated group (Group 4) the liver sections showed a slight vascular congestion and normal lobular architecture, similar to that in the normal control group **[Fig.2.(D)].**

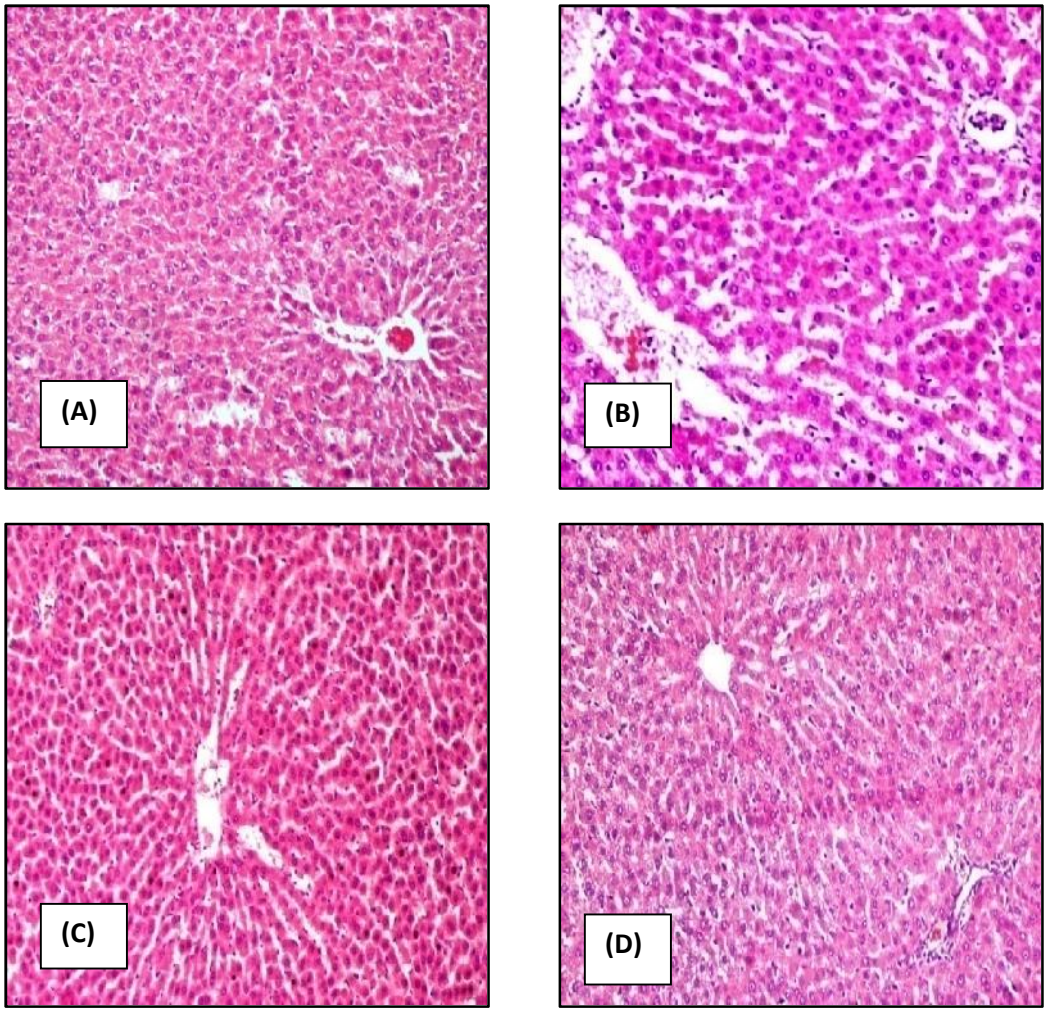

Fig. 2. Histopathological Studies of Liver in Anti-Tubercular Drugs (INH + RIF) induced Hepatotoxicity Model

(A) Normal Control (Group 1); (B) Negative Control (Group 2); (C) AECPL + INH + RIF (Group 3); (D) Silymarin + INH + RIF (Group 4)

4. DISCUSSION

The liver is responsible for processing most of the chemicals and medications that enter the body; thus leaving it vulnerable to acute or chronic liver diseases caused by chemicals. In some cases, this is a predictable consequence of over-consumption of certain chemicals such as acetaminophen and some prescription medications, including antibiotics, non-steroidal anti-inflammatory drugs and anti-convulsants; or due to exposure to industrial toxins like polyvinyl chloride or carbon tetrachloride (CCl_4). Other causes of liver diseases are excessive alcohol consumption, vascular diseases, rare metabolic diseases such as

Wilson's disease, obesity and congenital birth defects. The efficacy of any hepatoprotective drug is essentially dependent on its capability to either reduce the harmful effects or to maintain the normal hepatic physiological mechanisms which has been unbalanced by the hepatotoxins.

Carica papaya is a tropical plant belonging to family Caricaceae. The *Carica papaya* fruit [16] and seeds [17] have been scientifically proved in animals for its significant hepatoprotective activity against CCl_4. The aqueous extract of leaves of *C.papaya* at the doses of 200 and 400 mg/kg (p.o.) has been evaluated earlier for its hepatoprotective activity against CCl_4- induced hepatotoxicity in rats [5]. Results showed that the extract caused significant and dose-dependent decrease in the serum levels of SGPT, SGOT, ALP, bilirubin and serum MDA. It was also observed that maximum hepatoprotection was offered at the oral dose of 400 mg/kg of the extract. Venugopalan et al. [6] also studied the leaf extract of *C.papaya* at an oral dose of 400 mg/kg against paracetamol induced liver damage and found significant hepatoprotection. Thus, the earlier studies suggested that *C.papaya* possesses potent hepatoprotective activity in experimental animals.

The leaves of *Carica papaya* have however not been proved for its efficacy against other important hepatotoxins. Thus, the purpose of the present study was to evaluate the hepatoprotective activity of the aqueous extract of leaves of *Carica papaya* against ethanol induced- and anti-tubercular drug induced-liver damage in rats. The dose selected for the present study was 400 mg/kg (p.o.) as this dose has been proved to be the most effective hepatoprotective dose against the heptotoxicants studied earlier.

The metabolic activation and biochemical mechanisms of hepatotoxicity induced by ethanol have been reviewed earlier [18]. Ethanol produces a constellation of dose-related deleterious effects in the liver. Alcohol ingestion is documented to cause fatty infiltration, hepatitis and cirrhosis. Oxidative stress is one major factor in the etiology of ethanol induced injury, mainly by Kupffer cell derived reactive oxygen species (ROS). Ethanol activates the Kupffer cells primarily through the action of a substance called endotoxin, which is released by certain gram negative bacteria present in the intestine. The activation of Kupffer cells generates ROS and proinflammatory cytokines (TNF alpha, IL-1), both leading to liver

damage. Hepatitis and cirrhosis may occur due to higher lipid peroxidative reaction during the microsomal breakdown of ethanol. Increased formation of lipoperoxides, conjugated dienes and malondialdehyde (MDA) and reduced levels of antioxidants like vitamin E and glutathione in the tissues have been demonstrated in experimental animals administered with ethanol as well as alcoholic human subjects. In the present study, a single dose administration of ethanol (4.5 ml/kg, p.o.) caused a significant ($P < 0.001$) increase in the levels of SGPT, SGOT, ALP and total bilirubin, serum indicators of hepatic damage. It was also accompanied by an increase in the level of tissue TBARS and marked reduction in the levels of GSH and SOD which indicated the presence of oxidative stress.

Anti-tubercular drugs like Isoniazid (INH) and Rifampicin (RIF) when given in combination enhances their toxic effect. Hepatocytes of the INH + RIF generally show liver cell necrosis and inflammation in experimental animals [19]. INH is metabolized to mono-acetyl hydrazine (AcHz), which is further metabolized to a toxic product by cytochrome P450, leading to hepatotoxicity. Patients on concurrent rifampicin therapy have an increased incidence of hepatitis. This has been postulated due to rifampicin-induced cytochrome P450 enzyme induction, causing an increased production of the toxic metabolites from acetyl-hydrazine (AcHz). Rifampicin also increases the metabolism of INH to isonicotinic acid and hydrazine, both of which are hepatotoxic. The plasma half life of AcHz (metabolite of INH) is shortened by rifampicin and AcHz is quickly converted to its active metabolites by increasing the oxidative elimination rate of AcHz, which is related to the higher incidence of liver necrosis caused by INH and rifampicin in combination. Rifampicin is also known to induce the hydrolysis pathway of INH metabolism into the hepatotoxic metabolite hydrazine [20]. The generation of oxidative stress was also found to play a role in liver damage induced by anti-tubercular drugs. In the present study too, administration of combination of INH + RIF at the dose of 125 mg/kg (i.p.) for 10 days to rats showed significant ($P < 0.001$) increase in the levels of hepato-specific enzymes like SGPT, SGOT, ALP and total bilirubin when compared to the normal control group, indicating liver damage. Similarly, increase in the extent of lipid peroxidation (TBARS) and reduction in the GSH level indicated the presence of oxidative damage.

In the present study, treatment of rats with aqueous extract of leaves of *Carica papaya* (AECPL) at the dose of 400 mg/kg (p.o.) in both the animal models significantly ($P < 0.001$) decreased the levels of

SGPT, SGOT, ALP and total bilirubin in the serum, which indicated its hepatoprotective effect. Also the extract was found to reduce the level of TBARS and increase the levels of GSH and SOD, revealing the antioxidant nature of the extract.

Silybum marianum (Milk thistle; Family: Asteraceae/Compositae) is one of the oldest and thoroughly researched plants in the treatment of liver diseases. It has been proved for its effectiveness against ethanol- induced hepatotoxicity model in rats [21] and antitubercular drugs induced hepatotoxicity [22]. Recently, several studies have been carried out to elucidate the mechanism of action of silymarin. Accumulated data show that this herbal drug potentiates the antioxidant capacity of the liver, acts as a scavenger of oxygen free radicals [23], inhibits the synthesis of pro-inflammatory cytokines [24], in addition to its hepatoprotective actions. In the present study, silymarin administration in rats at the dose of 200 mg/kg significantly ($p<0.001$) lowered the levels of serum liver markers (SGPT, SGOT, ALP and total bilirubin); and increased the antioxidant parameters (GSH and SOD) while it reduced the level of TBARS in the liver tissue. Also, the effects produced by 400 mg/kg of aqueous extract of *Carica papaya* leaves were found to be comparable to that of the standard drug, silymarin (200 mg/kg, p.o.).

The results were further confirmed by histopathological studies. The liver sections of animals exposed to hepatotoxicants, namely ethanol and anti-tubercular drugs showed histological changes which were similar to those reported earlier by many researchers. In the animals treated *Carica papaya* or Silymarin, the hepatocytes showed restoration or preservation of the normal tissue architecture. This proved the ameliorative effect of *Carica papaya* leaves against ethanol-induced and anti-tubercular drugs induced hepatotoxic changes.

In the present study, preliminary phytochemical studies of the aqueous extract of leaves of *Carica papaya* showed the presence of flavonoids, alkaloids and saponins. Literature review revealed that the hepatoprotective activity of herbal drugs is due to the presence of different chemical constituents like flavonoids [25, 26], alkaloids [27], triterpenoids [28], and saponins [29]. It has also been reported that flavonoids are able to inhibit drug-induced hepatotoxicity in experimental models due to their potent anti-oxidant or free radical scavenging properties [30, 31]. In addition, literature [32] has shown that alkaloids

elicit hepatoprotective activity by strongly inhibiting lipid peroxidation (by reduction of malondialdehyde production) and cell membrane disruption (reduction of lactate dehydrogenase leakage induced in isolated hepatocytes). The leaves of *Carica papaya* also contain vitamin C (ascorbic acid) and carotene, which are well documented for their antioxidant activities [33]. These antioxidants along with flavonoids and alkaloids may have counteracted the free radicals through effective scavenging.

Also, vitamin C has been proved for its liver protective actions against various hepatotoxicants like thioacetamide-induced cirrhosis model in rats [34] and lead-induced liver damage [35]. Recently, Dutta et al. [36] have also proved the protective role of ascorbic acid (Vit C) against alcohol induced hepatotoxicity. This thus indicates that the presence of these natural antioxidants and hepatoprotective substances in *Carica papaya* leaves may play an important role in hepatoprotection.

5. CONCLUSION

The study thus concludes that the leaves of *Carica papaya* possess hepatoprotective activity, which may be partly due to its antioxidant effect. It also suggests that *Carica papaya* leaves are a promising candidate for the development of phytomedicine and can be used in herbal formulations alone or in combination with other hepatoprotective drugs (to provide a synergistic effect) for the treatment of liver ailments. Further, studies in humans are needed in this direction to prove its clinical utility.

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COMPETING INTERESTS

The authors declare no competing interest.

AUTHORS' CONTRIBUTIONS

Mr. Aashish Pandit did the literature survey, designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Mr. Tarun Sachdeva helped Mr. Aashish to perform the study and carry out the analysis of the data. Dr. Pallavi Bafna helped in designing the study and checking the manuscript. All authors have read and approved the final manuscript.

CONSENT

Not applicable

ETHICAL APPROVAL

Experiments have been examined and approved by the Institutional Animal Ethical Committee (IAEC) and have therefore been performed in accordance with the ethical standards.

REFERENCES

[1] Bell BP, Navarro VJ, Manos MM, Murphy RC, Leyden WA, St. Louis TE, et al. The epidemiology of newly diagnosed chronic liver diseases in the United States: Findings of population-based sentinel surveillance. Hepatol. 2001;34:468A.

[2] Ezzati M, Lopez A, Rodgers A, Vander-Hoorn S, Murray C. Selected major risk factors and global and regional burden of disease. Lancet. 2002;360:1347-60.

[3] World Health Organization. Global status report on alcohol 2004. Geneva, Switzerland; 2004.

[4] Girling DJ. The hepatic toxicity of antituberculosis regimens containing isoniazid, rifampicin and pyrazinamide. Tubercle. 1978;59:13-32.

[5] Mohammed A, Abubakar SA, Sule MS. Hepatoprotective effect of aqueous leaf extract of *Carica papaya* Linn against CCl_4 induced hepatic damage in rats. Int J Pharm Sci Rev Res. 2011;11:13-6.

[6] Venugopalan R, Perumal P, Gaddam R, Rajagoundan R. Hepatoprotective and *in vivo* antioxidant activity of methanol extract of *Carica papaya* Linn. leaves on paracetamol -induced liver damage in rats. J Pharm Res. 2012;5:3085-91.

[7] Trease GE and Evans WC. A Text book of Pharmacognosy. 13th ed. London: Bailliere Tindall Ltd.; 1989.

[8] Khandelwal KR. Practical Pharmacognosy techniques and experiments. 12th ed. Pune: Nirali Prakashan; 2004.

[9] Zhang P, Bagby GJ, Xie M, Stoltz DA, Summer WR, Nelson S. Acute ethanol intoxication inhibits neutrophil beta 2-integrin expression in rats during endotoxemia. Alcohol Clin Exp Res. 1998;22:135-41.

[10] Jiang Y, Renxiu P, Jing Y, Rui K, Juan L. CYP2E1 mediated Isoniazid-induced hepatotoxicity in rats. Acta Pharmacol Sin. 2004;25:699-704.

[11] Saleem MTS, Christina AJM, Chidambaranathan N, Ravi V, Gauthaman K. Hepatoprotective activity of *Annona squamosa* on experimental animal model. Int J Appl Res Nat Pro. 2008;1:1-7.

[12] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351-8.

[13] Beutler E, Duron O, Kellin BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963;61:882-8.

[14] Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;247:3170-5.

[15] Lowry OH, Rosebrough NH, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Bio Chem. 1951;193:265-75.

[16] Rajkapoor B, Jayakar B, Kavimani S, Murugesh N. Effect of dried fruits of *Carica papaya* Linn. on hepatotoxicity. Biol Pharm Bull. 2002;25:1645-6.

[17] Adeneye AA, Olagunju JA, Banjo AAF, Abdul SF, Sanusi OA, Sanni OO, et al. The aqueous seed extract of *Carica papaya* Linn prevents carbon tetrachloride induced hepatotoxicity in rats. Int J App Res Nat Prod. 2009;2:19-32.

[18] Tsukamoto H, Kaplowitz N. Pathogenesis of alcoholic liver disease. In: Liver and Biliary Diseases, Kaplowitz N, editor. Baltimore: Williams and Wilkins; 1996.

[19] Ravi V, Patel SS, Verma NK, Dutta D, Saleem TSM. Hepatoprotective Activity of *Bombax ceiba* Linn against Isoniazid and Rifampicin-induced Toxicity in Experimental Rats. Int J App Res Nat Prod. 2010;3:19-26.

[20] Padma VV, Suja V, Shyamala DCS. Hepatoprotective effect of Liv-52 on antitubercular drug-induced hepatotoxicity in rats. Fitoterapia. 1998;69:520-2.

[21] Das SK, Vasudevan DM. Protective effects of silymarin, a milk thistle (*Silybium marianum*) derivative on ethanol-induced oxidative stress liver in liver. Ind J Biochem Biophys. 2006;43:306-11.

[22] Bansal S, Tekur U, Chaturvedi UK, Gupta VK. Hepatoprotective Effect of silymarin against hepatotoxicity induced by anti-tubercular drugs in malnourished rats. The Faseb J. 2007;21:729.

[23] Lu ZM, Tao WY, Zou XL, Fu HZ, Ao ZH. Protective effects of mycelia of *Antrodia camphorata* and *Armillariella tabescens* in submerged culture against ethanol-induced hepatic toxicity in rats. J Ethnopharmacol. 2007;110:160-4.

[24] Huang GJ, Deng JS, Chiu CS, Liao JC, Hsieh WT, Sheu MJ, et al. Hispolon protects against acute liver damage in the rat by inhibiting lipid peroxidation, proinflammatory cytokine, and oxidative stress and down regulating the expressions of iNOS, COX-2, and MMP-9. Evid-Based Compl Alt Med. 2012;2012:1-12.

[25] Paya M, Ferrandiz ML, Sanz MJ, Alcaraz MJ. Effects of phenolic compounds on bromobenzene mediated hepatotoxicity in mice. Xenobiotica. 1993;23:327-33.

[26] Carini R, Comoglio A, Albano E, Poli G. Lipid peroxidation and irreversible damage in the rat hepatocytes model: Protection by the silybin-phospholipid complex IdB 1016. Biochem Pharmacol. 1992; 38: 2859-2865.

[27] Manjunatha BK, Mankani KL, Vidya SM, Krishna V, Manohara YN. Hepatoprotective activity of *Butea superba* against carbon tetrachloride induced hepatic damage in rodents. Pharmacog Mag. 2008;4:41-5.

[28] Gao J, Tang X, Dou H, Fan Y, Zhao X, Xu Q. Hepatoprotective activity of *Terminalia catappa* Linn. leaves and its two triterpenoids. J Pharm Pharmacol. 2004;56:1449-55.

[29] Tran QL, Adnyana IK, Tezuka Y, Nagaoka QK, Tran, Kadota S. Triterpene saponins from Vietnamese ginseng (*Panax vietnamensis*) and their hepatocyte protective activity. J Nat Prod. 2001;64:456-61.

[30] Fraga CG, Martino VS, Ferraro GE, Coussio JD, Boveris A. Flavonoids as antioxidants evaluated by *in vitro* and *in situ* liver chemiluminiscence. Biochem Pharmacol. 1987;36:717-20.

[31] Laughton MJ, Halliwell B, Evans PJ, Hoult JRS. Antioxidant and pro-oxidant actions of the plant phenolics quercetin, gossypol and myristicin effects on lipid peroxidation, hydroxyl radical generation and bleomycin-dependent damage to DNA. Biochem Pharmacol. 1989;38:2859-65.

[32] Lanhers MC, Joyeux M, Soulimani R, Fleurentin J, Sayag M, Mortier F, et al. Hepatoprotective and anti-inflammatory effects of a traditional medicinal plant of Chile, *Pneumus boldus*. Planta Med. 1991;57:110-5.

[33] Sies H, Stahl W, Sundquist AR. Antioxidant functions of vitamins: Vitamins E and C, beta-carotene, and other carotenoids. Ann N Y Acad Sci. 1992;669:7-20.

[34] Atef MAA. Hepatoprotective influence of vitamin C on thioacetamide-induced liver cirrhosis in wistar male rats. J Pharmacol Toxicol. 2011;6:218-33.

[35] Ajayi GO, Adeniyi TT, Babayemi DO. Hepatoprotective and some haematological effects of *Allium sativum* and vitamin C in lead-exposed wistar rats. Int J Med Med Sci. 2009;1:64-7.

[36] Datta G, Basu S, Sen A, Nath P. Role of α-tocopherol, ß-carotene and ascorbic acid against alcohol induced hepatotoxicity: A comparative analysis. J Pharm Res. 2012;5:2485-8.