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Protective effect of curcumin and picroliv on chronic alcoholic liver cirrhosis of rat induced with low caloric diet

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Abstract: The present investigation aims at evaluation of the protective efficacy of picroliv and curcumin alone and combination against chronic ethanol exposure induced liver damage. Alcoholic liver cirrhosis was induced in the rat by low calorie diet feeding along with oral ethanol intake in gradually increasing concentration (25-75%) over the duration of 35 days (5 weeks). Picroliv (30 mg/kg) and curcumin (70 mg/kg) were given orally 2 h after the ethanol administration starting from the fifth week, continuously for seven days. On 35th day animals were sacrificed, blood and organ samples were collected, and SGOT, SGPT, ALP, cholesterol (TC), triglyceride (TG), bilirubin, total protein, and albumin were measured. Curcumin alone and in combination with picroliv showed a reversal of body weight loss induced by alcohol. Picroliv, curcumin, and the combination of picroliv and curcumin significantly ($P < 0.001$) reduced SGOT, SGPT, ALP, TC, and TG. Histopathology of liver section showed mostly normal hepatocytes with the absence of necrosis and cirrhosis in the combination treated group. Picroliv and curcumin combination significantly reduced hyperlipidemia, hyperbilirubinemia, replenish albumin loss, reversed cirrhosis, necrosis and inflammatory changes in the liver may be due to free radical scavenging, antioxidant and membrane stabilizing properties suppressing alcohol induced expression of cytokines and chemokines.

Keywords: Alcohol, Curcumin, Liver cirrhosis**,** Low calorie diet, Picroliv.

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I. INTRODUCTION

The liver performs an astonishingly large number of tasks including vascular, secretory, excretory and metabolic functions controlling synthesis and utilization of carbohydrates, lipids, and proteins. Liver disorders can result from a wide variety of insults, including infections, drugs, toxins, ischemia, and autoimmune disorders. Most liver disorders produce hepatocellular injury and necrosis of some degree, resulting in various abnormal laboratory test results and symptoms. Chronic consumption of alcohol can cause a spectrum of liver abnormalities, ranging from fatty liver to steatohepatitis, cirrhosis and hepatocellular carcinoma. Patients with alcohol related steatosis rarely manifest symptoms suggestive of liver disease and are usually identified incidentally from abnormal blood test results (McClain and Beier, 2010). There is no cure for available alcoholic cirrhosis, treatment can stop or slow down the progression of the liver damage and helps to reduce the complications. Alcoholic liver disease includes acute alcoholic hepatitis and alcoholic cirrhosis, is a major cause of morbidity and mortality in the Western world and in India also alcohol is most commonly abused substance. Treatment of acute alcoholic injury is nutritional support, corticosteroids, anti-inflammatory drugs and antioxidants, and agents that are directed against the progression to fibrosis, such as colchicines, propylthiouracil, and antioxidants. These therapies are inefficient to alter the process of injury and to repair the liver, thus liver transplantation remains the ultimate option for patients with liver failure due to chronic alcoholic liver damage (Tome and Lucey, 2004).

Therapeutic exploration of herbal products with the modern concept of evaluation and standardization has shown promising results against hepatic diseases. Milk thistle, dandelion, licorice, chicory, and kutki are some of the most commonly used herbs for liver problems (Zafar and Ali, 1998). Kutki is the common name of the herb *Picrorrhizakurroa*Benth. (family: Scrophulariaceae) found in the Himalayas from Kashmir to Sikkim at the altitude of 2700-4500 m. The main iridoid glycoside reported from rhizomes of *P. kurroa* is picroliv (Chander et al., 1990) or kutkin which is a mixture of picroside I and kutkoside and is primarily responsible for hepatoprotective activity (Shukla et al., 1991; Dwivedi et al., 1992). P. kurroa has been shown to protect the liver from a wide variety of insults including galactosamine, ethanol and aflatoxin B1 (Dwivedi et al., 1993a; Dwivedi et al., 1993b; Rastogi et al., 1996). Picroliv treatment effectively reverses ethanol induced and

ischaemic liver damage by improving hepatocyte glycogen preservation and reduced apoptosis (Saraswat et al., 1999; Singh et al., 2000).

Curcuma longa Linn (Family: Zingiberaceae) is a small perennial herb native to India bearing rhizomes which are the source of culinary spice known as Turmeric. The active constituents of turmeric are the flavonoid curcumin (diferuloylmethane), which comprises 0.3-5.4 percent of raw turmeric. Curcumin attenuates liver injury induced by acute ethanol, thioacetamide, paracetamol, iron overdose, cadmium and carbon tetrachloride intoxication (Reddy and Lokesh, 1996; Park et al., 2000; Nanji et al., 2003; Eybl et al., 2004; Samuhasaneeto et al., 2009; Wang et al., 2012; Somanawat et al., 2013).

Picroliv and curcumin protect the liver from a wide variety of insults and have enormous commercial potential throughout the globe. In previous studies, picroliv and curcumin both have effectively reversed ethanol induced liver damage along with their antioxidant potential. The present investigation aims at evaluation of the protective efficacy of picroliv and curcumin alone and combination against chronic ethanol exposure induced liver damage. The experimental animals were given low carbohydrate diet along with increasing amount of ethanol to cause hepatocellular fibrosis as alcohol administration per se was not able to injure the liver beyond fatty infiltration in rats possibly because of a short lifespan. Picroliv and curcumin were explored as a promising therapy for treating alcoholism related liver cirrhosis.

II. MATERIALS AND METHODS

2.1 Plant material

P. kurroa rhizomes were procured from Khari Bawli market, Delhi, India in the month of November 2008. The plant was identified with the aid of available literature and authenticated by taxonomist Dr. H. B. Singh of Herbarium Department, NISCAIR, New Delhi (voucher specimen no. NISCAIR/RHMD/consult/-2009-10/1233/37).

2.2 Preparation of methanolic extract

The plant material was dried in a tray dryer at temperature 45ºC±2ºC, milled into the coarse powder and passed through the sieve 40/60. The coarse powder (250 g) was soaked in 1.0 l of 95% ethanol for four days with intermittent shaking. On the 5th day, the whole material was filtered through muslin cloth. The filtrate was collected and concentrated. The residual solvent was removed under vacuum in a rotatory vacuum evaporator under 40ºC at 650 mm vacuum pressure and a solid blackish-brown mass was obtained (yield: 9.30% w/w).

2.3 Isolation of picroliv

The solid mass was pulverized to a fine powder of mesh 80-100, dissolved in acetone and warmed at 50ºC. This mixture was poured in 80% acetone with continuous stirring for 1 h and set aside for 2 h. The clear supernatant liquid was filtered; the process was repeated for six times. The different fractions of acetone were mixed and evaporated under reduced pressure at 50ºC and the dried residue obtained was picroliv (yield: 7.09% w/w). This isolated picroliv was used for the experimental studies.

2.4 Authentication of isolated picroliv

2.4.1 Confirmatory test for picroliv

Picroliv was tested with Godin's reagent for colour development as described by MacLennan *et al*. (1959).

2.4.2 High Performance Liquid Chromatography (HPLC)

The HPLC system (Shimadzu, Japan), equipped with CAT-228-39001-38 pump, 228-393000-38 photodiode array detector, LC solution integrated software and a Rheodyne injection valve fitted with a 20 µl injection loop, was used for the analysis. Baseline resolution of picroliv was obtained at 25±2ºC using stainless steel column (15 cm × 4.6 mm), packed with octadecylsilane bonded to porous silica (5 µm). An isocratic solvent system consisting of 1% v/v of orthophosphoric acid: acetonitrile in the ratio of 83:17 (v/v) was used. The mobile phase was passed through 0.45 PVDF filter, degassed before use. The flow rate was kept constant at 1 ml/m and effluents were monitored at 280 nm. The test solution was prepared by dissolving 100 mg of the substance under examination in 25 ml of methanol and filtered (Indian Pharmacopoeia, 2007).

2.5 Authentication of curcumin

2.5.1 Curcumin source

Curcumin was purchased from KuberImex, Indore (M.P.).

2.5.2 Determination of λ_{max}

Standard solution (10 µg/ml) of pure curcumin was prepared in methanol. The drug solution was scanned on UV spectrophotometer (Shimadzu 1800, Japan) between 200 to 800 nm.

2.5.3 HPLC

The mobile phase consisting of acetonitril: tetrahydrofuran: 2% acetic acid in a ratio of 50:30:20 (v/v) was used. The flow rate was maintained at 1.0 ml/m and the effluent was monitored 425 nm. Curcumin sample solution was prepared by dissolving 10 mg in 100 ml of mobile phase and filtered. The injection volume was 50 µL and analysis was performed at ambient temperature following the method of Yadavand Sarasija(2009).

2.6 In vivo study

2.6.1 Test animals

Laboratory bred Wistar albino rats of either sex weighing between 150-200 g were maintained under standard laboratory conditions at 25±2ºC, relative humidity 50±15% and photoperiod (12 hdark and light). During pre-experimental period laboratory prepared control diet (Table 1) and water were provided *ad libitum*. Animals were allowed to free access to water and food during the experiment but no water and food were allowed before and after one h of dosing. In order to avoid diurnal variation, all the dosing were carried out at the same time of day i.e. between 10:00 am to 05:00 pm. Ethical committee approval was obtained from Institutional Animal Ethical Committee (Reg. no. 1169/ac/08/CPCSEA), the approved body of Committee for the Purpose of Control and Supervision of Experiments on Animals, Chennai, India.

2.6.2 Dose selection

Picroliv was administered at 30 mg/kg dose as previously reported by us the optimal hepatoprotective dose of picroliv is 20 to 30 mg/kg(Bigoniya et al., 2010). Curcumin oral dose for oral administration was selected based on published reports of Menozzi*et al*.(2009) and Hsieh*et al*. (2014).

2.6.3 Low calorie diet

Alcoholic liver cirrhosis was induced in the rat by low calorie diet feeding along with oral ethanol intake in gradually increasing concentration (25-75%) over the duration of 35 days (5 weeks). Low calorie diet was prepared as food pellets following the standard composition is given in Table 1. Low calorie diet feeding assumes the average food intake of 10 g/day/100 g body weight of animals (Fisher et al., 1996). Rats were weighed and randomly divided into 6 groups of six rats each.

2.6.4 Group division

Group I	Low calorie diet control
Group II	Low calorie diet + Ethanol (25%-75%, p.o)
Group III	Low calorie diet + Ethanol (25%-75%) + Silymarin (20 mg /kg, p.o)
Group IV	Low calorie diet + Ethanol (25%-75%) + Picroliv (30 mg/kg, p.o)
Group V	Low calorie diet + Ethanol (25%-75%) + Curcumin (70 mg /kg, p.o)
Group VI	Low calorie diet + Ethanol (25%-75%) + Picroliv (30 mg/kg) + Curcumin (70 mg /kg, p.o)

The alcohol composition were increase gradually from 25% to 75% by following previous experimental hepatotoxicity induction as reported by Nakajima*et al*. (2004) with slight modification(Nakajima et al., 2004).

2.6.5 Treatment plan

1st-2nd week	Low calorie diet
3rd week	Low calorie diet + 25% ethanol (2 ml/kg) for 4 days
	Low calorie diet + 25% ethanol (4 ml/kg) for 3 days
4th week	Low calorie diet + 50% ethanol (2 ml/kg) for 4 days
	Low calorie diet + 50% ethanol (4 ml/kg) for 3 days
5th week	Low calorie diet + 75% ethanol (2 ml/kg) for 4 days
	Low calorie diet + 75% ethanol (4 ml/kg) for 3 days

The different doses of experimental and standard drugs were given 2 h after the ethanol administration in the fifth week of the study, continuously for seven days.

2.6.6 Hepatoprotective effect assessment

Body weight was measured on every 7th day throughout the study period and percentage change was calculated on weekly basis. On 35th day animals were anaesthetized, sacrificed and blood sample was collected by heart puncture. The organs (liver, pancreas, kidney, heart) were isolated and kept in saline, washed and weighed. Serum was separated after coagulating at 30°C for 30 m and centrifuged at 3000 rpm for 15 m. Serum was analyzed for various biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total cholesterol (TC), triglyceride (TG), bilirubin, total protein and albumin as per the standard method using diagnostic kit (Span Diagnostic Ltd,

Surat).

2.6.7 Histopathology of liver

Preparation of permanent liver tissue slides and staining (haematoxylin and eosin) was based on the method of Nanji*et al*.(2002).

III. RESULTS

3.1 Authentication of isolated picroliv

Picroliv (m.p. 210ºC) was obtained as brown crystalline powder having bitter taste. It exhibited a red purple color with Godin's reagent(MacLennan et al., 1959).The isolated picroliv was further authenticated by HPLC analysis. The HPLC method used herein provides a good separation of picroliv. Chromatogram of picroliv reference standard(Indian Pharmacopoeia, 2007) and chromatogram of isolated picroliv showed identical peaks and represented in Fig.1Aand 1B. Under the chromatographic conditions, the retention time of the isolated picroliv was about 13.80 m identical to that of picroliv reference standard(Fig. 1).

3.2 Authentication of curcumin

UV absorption maxima of curcumin are reported to be 419 nm by Pandit*et al*. (2015),which is near to the observed ƛ max at 420 nm (Fig. 2).The mobile phase containing acetonitrile,tetrahydrofuro, 2% acetic acid (50:30:20v/v)showed well defined and resolved HPLC peak with retention time 4.017 m as compared to reported retention time 4.587m in Yadavand Sarasija(2009) as shown in Fig. 3.

3.3 Effect on body weight

Body weight of all the animals was measured on first day before commencing the experiment then on weekly basis.The animals feed with LCD showed 6.93% decrease whereas LCD along with ethanol showed 16.09% loss in body weight on 35th day. Standard drug silymarin, picroliv, curcumin, and combination of picroliv and curcumin showed respectively 9.75%, 12.88%, 9.19%, and 8.24%decrease in body weight on 35th day (Table 2).

3.4 Effect of on organ weight

Simultaneous feeding of LCD and ethanol significantly ($P< 0.01$) increased the liver weight but kidney, heart and spleen were unaffected. Silymarin, picroliv, and combination of picroliv and curcumin significantly ($P< 0.01$) reduced ethanol induced elevated liver weight. Curcumin also reduced ($P< 0.05$) liver weight but was less effective compared to the other counterparts (Table 3).

3.5 Effect on biochemical parameters

Combination of LCD and ethanol treated group showed significantly ($P< 0.001$) elevated serum level of SGOT, SGPT, ALP, TC, TG and bilirubin.LCD and ethanol treatment also showed significant ($P< 0.05$-0.01) reduction in the level of total protein and albumin. Significant reduction of SGOT, SGPT, ALP, TC and TG was observed in silymarin ($P< 0.001$), picroliv ($P< 0.001$), curcumin ($P< 0.01$-0.001), and combination of picroliv and curcumin ($P< 0.001$) treated groups. Silymarin, picroliv and curcumin were ineffective in reducingbilirubin level and also elevating level of total protein and albumin. Combination of picroliv and curcumin significantly reduced bilirubin ($P< 0.001$) and increased albumin ($P< 0.05$) but have no effect on the reduced level of total protein (Table 4).

3.6 Effect on liver histology

Light microscopic examination of liver section of the LCD control group showed normal histology. LCD and ethanol exposed liver showed abscess, cirrhosis, necrosis, inflammatory changes, sinusoidaldamage and hemorrhage. Silymarin showed moderate hepatic damage with absence of inflammation and abscess formation. Picrolivshowed protection with few focal necrotic site and presence of hemorrhage, inflammatory neutrophilic change. Curcumin showed normal hepatic vein and sinusoids with presence of focal necrotic damage. Picroliv and curcumin combination showed mostly normal hepatocytes with absence of necrosis and cirrhosis and presence of neutrophilic infiltration and sinusoid dilation(Fig. 4).

IV. DISCUSSION

Cirrhosis is the damage of liver cells and their gradual replacement with scar tissue that impairs blood flow through the liver causing hepatocyte death and loss of liver function (Wang et al., 2011). Liver cell damage induces inflammatory response which is accompanied by limited deposition of extracellular matrix (ECM). If the cells fail to regenerate during persistent liver injury, hepatocytes are replaced by abundant ECM including fibrillar collagen and hepatic fibrosis occurs after repeated injury (Friedman et al., 2002; O'Connell and Rushworth, 2008). Treatment options for common liver disease such as cirrhosis, fatty liver, and chronic

hepatitis are very less responsive. In alcoholics, poor nutrition is associated with an increased incidence and severity of liver disease (Antonow and McClain, 1985). Alcohol feeding in animals has been used as a model to imitate some of the features of the human liver disease (French, 1993). Alcohol fed rodents if maintained on diets containing low calorie develop fatty liver with inflammatory changes and fibrosis (Tsukamoto et al., 1985). Diets containing unusually low calorie content may cause overproduction of aldehydes. Alcohol induced oxidation of fatty acids liberates hydroxy-aldehydes that are believed to stimulate stellate cell proliferation and collagen deposition (Kamimura et al., 1992: Parola et al., 1993).

Looking in to the predominant role of oxidative stress in occurrence of liver cirrhosis, antioxidants are proposed as a treatment for cirrhosis (Loguercio and Federico, 2003). The effectiveness of treatments such as interferons, colchicines, penicillamine and corticosteroids are inconsistent and the incidences of side-effects are profound (Strader et al., 2004). Several studies have demonstrated the protective effects of antioxidants in liver injury by reducing oxidative stress in hepatocytes (Bansal et al., 2005; Cederbaum et al., 2009). A number of herbals show promising effect like; silymarin in liver cirrhosis, glycyrrhizin in chronic viral hepatitis, curcumin in tumor and picroliv in carcinogenesis of the liver (Stickel and Schuppan, 2007). Picroliv pretreatment has been reported to reduce hepatic ischemia-reperfusion injury by preserving hepatocyte glycogen and reducing apoptosis. Picroliv showed reduction in neutrophil infiltration, interleukin-1α and interleukin-1β level and increase in intracellular antioxidant enzyme superoxide dismutase. Tissue inflammatory cytokines level of interleukin-1alpha (IL-1alpha) and interleukin-1beta (IL-1beta) was also lower in picroliv group (Singh et al., 2000). Curcumin prevents paracetamol induced hepatitis through improvement of liver histopathology by decreasing oxidative stress, reducing liver inflammation and restoration of glutathione (Samuhasaneeto et al., 2009). Curcumin suppresses inflammatory responses and hepatic fibrogenesis by virtue of its cytoprotective effect (Wang et al., 2012). Considering the above facts, the current study was focused on finding new therapeutic solutions to minimize liver damage caused by alcoholism. Curcumin and picroliv individually and in combination were examined as a promising therapy for treating liver cirrhosis.

In the present study, the rat in the control and treated groups were administrated with the vehicle, LCD along with ethanol and treated with silymarin, picroliv, and curcumin alone and in combination. Alterations in body weight gain and internal organs weight ratio of animals reflect the toxicity after exposure to the toxic substances (Carol, 1995). The body weight changes are indicators of adverse effects of chemicals and are considered significant if the body weight gain or loss occurred is more than 10% from the initial weight (Raza et al., 2002; Teo et al., 2002). Administration of LCD alone and also in combination with ethanol significantly affected body weight by showing more than 10% suppression of body weight gain. Curcumin alone and in combination with picroliv showed a reversal of body weight loss induced by alcohol and maintained weight loss below 10%. Curcumin was more effective than picroliv in maintaining the reversal of weight loss caused by ethanol. Organ weight is also an important index of physiological and pathological status in animals. To assess, whether an organ has exposed to the injury or not the relative organ weight is a fundamental feature in diagnosis. The liver, kidney, spleen, and heart are the primary organs affected by the metabolic reaction caused by toxicant (Dybing et al., 2002). LCD with ethanol exposure has significantly increased liver weight in animals following 35 days of treatment. Treatment of picroliv, curcumin, and combination of picroliv and curcumin has normalized the liver weight. Picroliv was more effective in normalizing the ethanol induced increased liver weight signifying maintenance of intact defensive mechanism which has not allowed toxicant to accumulated in sufficient enough to manifest any deleterious change in the liver.

Alcohols are mostly metabolized in the liver through a series of chemical reactions known as oxidation reactions. The predominant biological pathway for alcohol metabolism is known as alcohol dehydrogenase pathway. The enzyme alcohol dehydrogenase converts alcohol to a toxic intermediate substance, acetaldehyde, then a second enzyme, aldehyde dehydrogenase, quickly converts acetaldehyde to acetate (Raza et al., 2002). The secondary pathway of alcohol metabolism is the microsomal ethanol-oxidizing system (MEOS), which involves the enzyme cytochrome P4502E1 or CYP2E1 activated by long-term heavy alcohol consumption (Dybing et al., 2002). In both of these pathways, more markedly in the MEOS pathway oxidation reactions spawn highly unstable free oxygen radicals.

Body deploys molecules called antioxidant to clear oxygen radicals from the liver. However heavy alcohol use not only heightens the production of oxygen radicals but also depletes the supply of antioxidants in the body, creating an imbalance between oxygen radicals and antioxidants. This imbalance is known as oxidative stress which damages liver cell membranes and mitochondria. When oxidative stress is chronic, it contributes to necrosis and liver fibrosis, in addition to this, oxidative stress appears to stimulate autoimmune reactions that further damage liver cells. The peroxidative products induce hypoperfusion of the membrane and finally, cytosolic enzymes SGOT, SGPT, ALP appear in the blood. Damaged liver cell plasma membrane starts to release lipoid substances like cholesterol, triglyceride, and bilirubin into the circulation, therefore these can be measured in serum as a marker of damage extent.

Low calorie diet given with gradually increasing concentration of ethanol for 35 days showed a severely elevated SGOT, SGPT, ALP, TC, TG, and bilirubin, whereas protein and albumin were decreased compared to normal. Results demonstrated that the picroliv, curcumin, and combination of both caused a significant reduction in the levels of elevated SGPT, SGOT, ALP, TC, and TG. Increase in serum level of ALP indicates increased synthesis function of hepatic cells in presence of increasing biliary pressure (Moss and Butterworth, 1974). The results of the experiment revealed that picroliv, curcumin, and combination of picroliv and curcumin caused a significant decrease in serum SGOT, SGPT, and ALP in comparison to negative control group indicating an improvement in the secretary mechanism of hepatic parenchyma and duct cells. Increase in cholesterol level in alcohol exposed group may be attributed to the inhibition or destruction of triglycerides secretary mechanism by the liver. Administration of picroliv, curcumin, and combination of both significantly reduced the level of total cholesterol and serum triglyceride that may be due to improvement in lipoprotein biosynthesis capacity in the animal of treated groups.

The rise in serum bilirubin or hyperbilirubinemia in LCD along alcohol treated rats indicates liver dysfunction. However, hyperbilirubinemia is not always present in well compensated cirrhosis and the presence of increased bilirubin is probably an indication of acute hepatitis induced by alcohol (Price and Alberti, 1985). Picroliv and curcumin alone were ineffective in reducing in the bilirubin level but in combination, they significantly prevented the severity of liver damage caused by alcohol as evidenced by the low level of bilirubin in the serum. This revealed the synergistic hepatoprotective role of picroliv and curcumin combination as the recovery of liver damage was at a significant level. Albumin is the most abundant protein in human plasma, representing 55-56% of total protein. It is synthesized in the liver at a rate that is dependent on protein intake subject to feedback regulation by the plasma level. Little albumin is filtered through the kidney glomeruli and most of it is reabsorbed by proximal tubule cells and degraded by lysosomal enzymes into fragments that are returned to the circulation. This study showed a decrease in serum albumin of rats treated with ethanol alone with LCD as compared to the only LCD control. This Indicates poor liver functions or impaired synthesis, either primarily as liver cells damage or secondarily to diminished protein intake and reduced absorption of amino acids caused by a malabsorption syndrome or malnutrition (Shibutani et al., 2001). Combination of picroliv and curcumin treatment increased serum albumin concentration in alcohol treated rats which reveals that picroliv and curcumin in combination can restore lipid peroxidation processes as well as is able to increase the activities of plasma protein thiols which is not observed when given individually (Al-Hashem et al., 2009). But the combination of picroliv and curcumin was ineffective in normalizing the total protein content in ethanol exposed animal.

Histopathology of liver section justifies the protective effect of picroliv and curcumin alone and also very predominantly when given in combination. Rats treated with low calorie diet with ethanol showed abscess, cirrhosis, necrosis, inflammatory changes, damage sinusoids, and hepatocytes. The liver section of animals treated with picroliv and curcumin alone showed some extent of hepatic vein damage, neutrophilic infiltration, and sinusoidal dilation but the absence of necrosis and cirrhosis with normal hepatocytes. The only abnormality present was slight inflammation around sinusoids in the combination treated group.

The various pathological and metabolic effects of ethanol are hyperlipemias, hyperuricemia, acetaldehyde toxicity and oxidative stress due to glutathione depletion (Lieber, 2000). The toxicity of alcohol is linked to its metabolism via alcohol dehydrogenase which converts nicotinamide adenine dinucleotide to reduced nicotinamide adenine dinucleotide, which contributes to hyperuricemia, hypoglycemia and hepatic steatosis by inhibiting lipid oxidation and promoting lipogenesis. This activity is also associated with the generation of free radicals with resulting lipid peroxidation and membrane damage as well as depletion of mitochondrial reduced glutathione (Lieber, 2005).

Picroliv has normalized several liver and serum biochemical parameters of albino rats following daily administration of ethyl alcohol for 45 days (Rastogi, 1996). Picroliv also restored the ethyl alcohol induced cholestasis as indicated by the increment in bile volume, bile salts and bile acids against ethanol induced hepatic injury in rats (Saraswat et al., 1999). NF-kappa B is a transcription factor which regulates genes involved in inflammation and is activated by endotoxin, cytokines and oxidative stress. Induction of NF-kappaB mediated gene expression has been implicated in the pathogenesis of the alcoholic liver disease. Curcumin blocked endotoxin-mediated activation of NF-kappaB and suppressed the expression of cytokines, chemokines, Cyclooxygenase-2 and nitric oxide synthase in Kupffer cells. Curcumin prevents experimental alcoholic liver disease, in part by suppressing induction of nuclear factor-kappaB-dependent genes (Nanji et al., 2003). Curcumin improved histopathology of the liver in the early stage of ethanol induced liver injury by reduction of oxidative stress and inhibition of nuclear factor-kappaB activation as reported by Samuhasaneeto et al. (2009).

V. CONCLUSION

In this study administration of picroliv and curcumin combination significantly reduced hyperlipidemia, hyperbilirubinemia and replenish albumin loss may be by restoring lipid peroxidation, improvement in lipoprotein biosynthesis and increasing plasma protein thiols activities. The combination most effectively reversed cirrhosis, necrosis and inflammatory changes in the liver could be due to free radical scavenging, antioxidant and membrane stabilizing properties of the curcumin and picroliv suppressing alcohol induced expression of cytokines and chemokines.

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Table 1: Composition of experimental low calorie diet

Components	Low calorie diet	Control diet
Casein	100 gm/kg	200 gm/kg
Corn Flour	534 gm/kg	350 gm/kg
Wheat bran	200 gm/kg	150 gm/kg
Dextrose	100 gm/kg	200 gm/kg
Cholesterol	2 gm/kg	4 gm/kg
Ghee	20 gm/kg	50 gm/kg
Vitamin	10 gm/kg	10 gm/kg
Mineral	30 gm/kg	30 gm/kg
Methionine-Dl	3 gm/kg	3 gm/kg
Salt	1 gm/kg	1 gm/kg

Table 2: Effect of picroliv and curcumin on percent change in body weight of low calorie diet feeding and alcohol ingestion induced liver cirrhosis on rats

Treatment (dose in mg/kg)	Average body weight in gm (% change)					
	0 day	7th day	14th day	21th day	28th day	35th day
LCD control	209.45 ± 4.50	204.67 ± 5.33 (-2.28%)	201.76 ± 5.06 (-3.67%)	198.97 ± 3.08 (-5.00%)	198.05 ± 3.89 (-5.44%)	194.93 ± 4.50 (-6.93%)
LCD + Ethanol	185.14 ± 3.22	180.17 ± 4.20 (-2.68%)	175.99 ± 4.30 (-4.94%)	172.47 ± 5.75 (-6.48%)	167.73 ± 3.56 (-9.40%)	155.35 ± 3.79 (-16.09%)
LCD + Ethanol + Silymarin (20)	201.46 ± 5.83	196.96 ± 4/04 (-2.23%)	194.70 ± 4.02 (-3.35%)	188.32 ± 4.35 (-6.52%)	183.24 ± 4.68 (-9.04%)	181.81 ± 4.37 (-9.75%)
LCD + Ethanol + Picroliv (30)	216.09 ± 5.01	209.52 ± 6.28 (-3.04%)	202.67 ± 6.70 (-6.21%)	195.36 ± 5.01 (-9.59%)	194.24 ± 3.85 (-10.11%)	188.25 ± 5.10 (-12.88%)
LCD + Ethanol + Curcumin (70)	198.07 ± 4.81	195.83 ± 6.22 (+2.24%)	191.58 ± 4.87 (-3.27%)	185.80 ± 4.58 (-6.19%)	181.74 ± 4.02 (-8.24%)	179.86 ± 4.22 (-9.19%)
LCD + Ethanol + Picroliv (30) + Curcumin (70)	191.89 ± 3.90	193.69 ± 4.93 (+1.80%)	186.25 ± 4.23 (-3.28%)	182.45 ± 3.55 (-5.49%)	180.73 ± 3.88 (-6.49%)	177.72 ± 4.38 (-8.24%)

n = 6 per group. LCD = Low calorie diet. All the data expressed as M ± SEM.

Table 3: Effect of picroliv and curcumin on percent change in organ weight of low calorie diet feeding and alcohol ingestion induced liver cirrhosis on rats

Treatment (dose in mg/kg)	Relative organ weight in gm/100 gm body weight			
	Liver	Kidney	Heart	Spleen
LCD control	4.03 ± 0.35	0.98 ± 0.032	0.33 ± 0.012	0.39 ± 0.011
LCD + Ethanol	6.66 ± 0.52**	0.81 ± 0.041^{ns}	0.31 ± 0.021^{ns}	0.41 ± 0.03^{ns}
LCD + Ethanol + Silymarin (20)	4.56 ± 0.41^{ns,b}	1.03 ± 0.012^{ns}	0.31 ± 0.013^{ns}	0.36 ± 0.012^{ns}
LCD + Ethanol + Picroliv (30)	4.46 ± 0.26^{ns,b}	0.95 ± 0.052^{ns}	0.34 ± 0.015^{ns}	0.40 ± 0.009^{ns}
LCD + Ethanol + Curcumin (70)	4.90 ± 0.22^{ns,a}	1.02 ± 0.020^{ns}	0.32 ± 0.026^{ns}	0.40 ± 0.006^{ns}
LCD + Ethanol + Picroliv (30) + Curcumin (70)	4.23 ± 0.45^{ns,b}	0.86 ± 0.062^{ns}	0.33 ± 0.013^{ns}	0.42 ± 0.003^{ns}

n = 6 per group. All the data expressed as M ± SEM. **< 0.01 compared to LCD control group. ^{a}< 0.05, ^{b}< 0.01 compared to LCD + ethanol group ns = not significant. LCD = Low calorie diet.

Table 4: Effect of picroliv and curcumin on serum biochemical parameter of low calorie diet feeding and alcohol ingestion induced liver cirrhosis on rats

Treatment (dose in mg/kg)	SGOT	SGPT	ALP	TC	TG	Bilirubin	Total protein	Albumin
LCD control	36.26 ± 2.38	39.56 ± 2.15	67.22 ± 3.02	105.22 ± 4.35	129.51 ± 10.16	0.33 ± 0.02	7.12 ± 0.52	5.05 ± 0.23
LCD + Ethanol	210.37 ± 10.30***	92.65 ± 3.60***	290.45 ± 12.86***	258.21 ± 13.81***	237.54 ± 12.02***	0.63 ± 0.02***	4.61 ± 0.35**	3.38 ± 0.16*
LCD + Ethanol+ Silymarin (20)	117.23 ± 0.16^{***,c}	55.91 ± 3.20^{*,c}	190.36 ± 8.09^{***,c}	143.60 ± 8.36^{*,c}	168.46 ± 9.04^{ns,c}	0.49 ± 0.014^{*,ns}	6.35 ± 0.37^{ns,ns}	4.97 ± 0.53^{ns,ns}
LCD + Ethanol+ Picroliv (30)	181.06 ± 9.50^{***,ns}	56.25 ± 4.06^{*,c}	129.30 ± 7.82^{***,c}	150.52 ± 7.02^{**,c}	150.50 ± 7.25^{ns,c}	0.48 ± 0.021^{ns,ns}	6.28 ± 0.47^{ns,ns}	3.84 ± 0.28^{ns,ns}
LCD + Ethanol+ Curcumin (70)	148.66 ± 8.52^{***,c}	49.07 ± 3.17^{ns,c}	95.32 ± 4.49^{ns,c}	118.53 ± 5.89^{ns,c}	185.32 ± 9.321^{**,b}	0.52 ± 0.030^{**,ns}	6.44 ± 0.40^{ns,ns}	4.53 ± 0.39^{ns,ns}
LCD + Ethanol+ Picroliv (30)+ Curcumin (70)	103.30 ± 6.39^{***,c}	34.06 ± 2.92^{ns,c}	78.96 ± 3.72^{ns,c}	109.75 ± 6.09^{ns,c}	134.54 ± 8.14^{ns,c}	0.37 ± 0.072^{ns,c}	7.45 ± 0.59^{ns,ns}	6.34 ± 0.46^{ns,a}

n = 6 per group. All the data expressed as M ± SEM. *< 0.05, **< 0.01, ***< 0.001 compared to LCD control group. ^{a}< 0.05, ^{b}< 0.01, ^{c}< 0.001 compared to LCD + ethanol group and ns = not significant. LCD = Low calorie diet, SGOT = serum glutamate oxaloacetate transaminase, SGPT = serum glutamate pyruvate transaminase, ALP = Alkaline phoaphatse, TC = Total cholesterol and TG = Triglycerides.

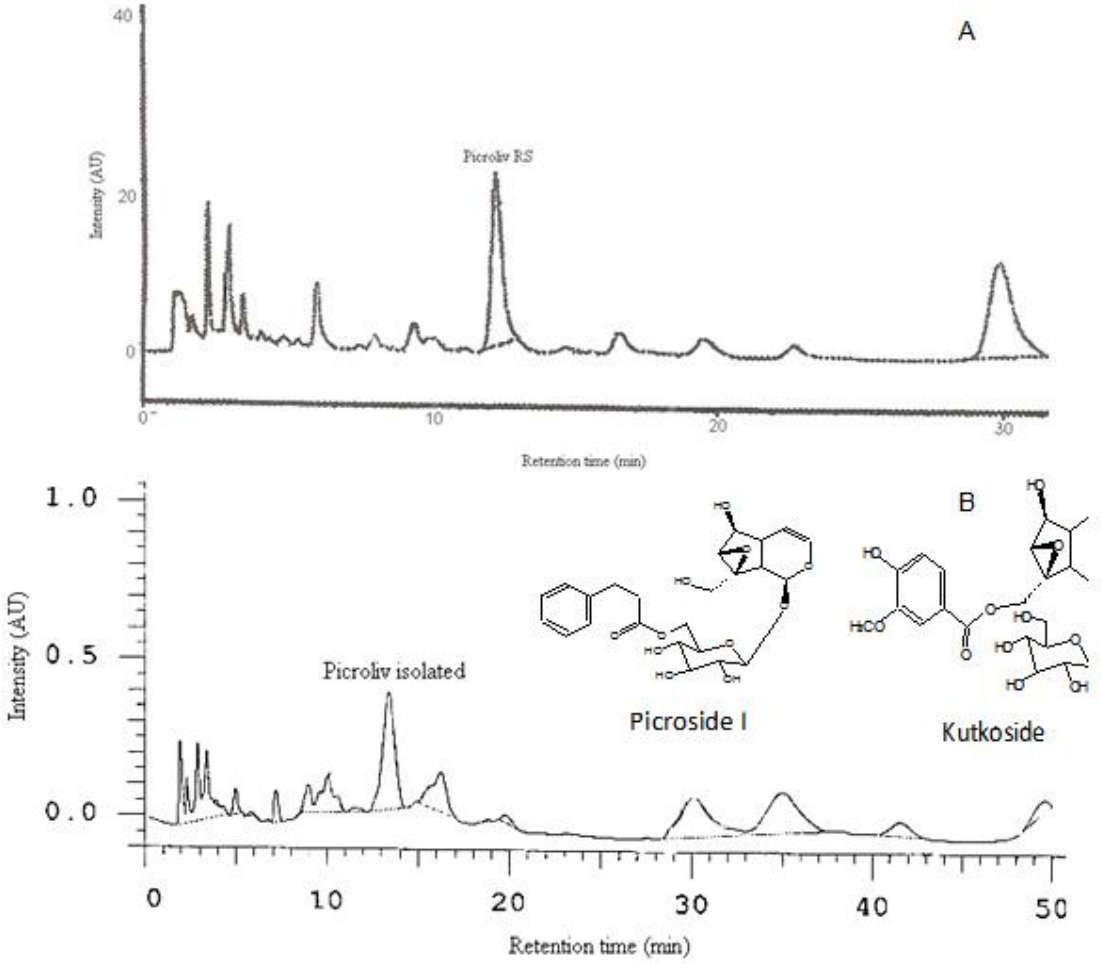

Fig. 1: HPLC chromatogram of picroliv. Standard picroliv (A) and isolated picroliv (B) in mobile phase 1% orthophosphoric acid in water and acetonitrile (83:17) scanned at 280 nm. Isolated picroliv showed retention time at 13.80 min identical with standard chromatogram reported in Indian Pharmacopoeia, 2007.

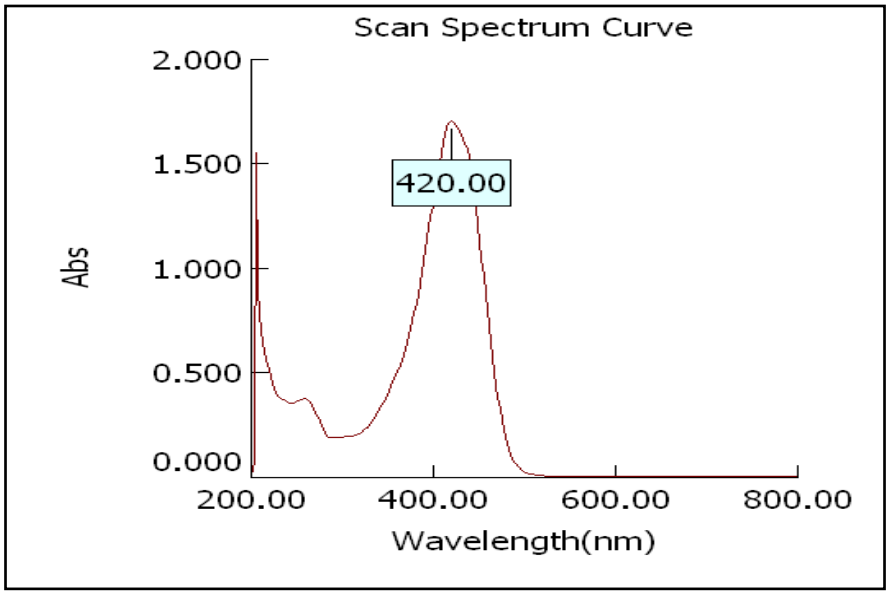

Fig. 2: UV spectrum of curcumin. Sample curcumin in methanol at 10 µg/ml concentration screened at 200 to 800 nm showedλ_{max} at 420 nm as compared to reported UV max 421 nm in Sharma *et al.*, 2012.

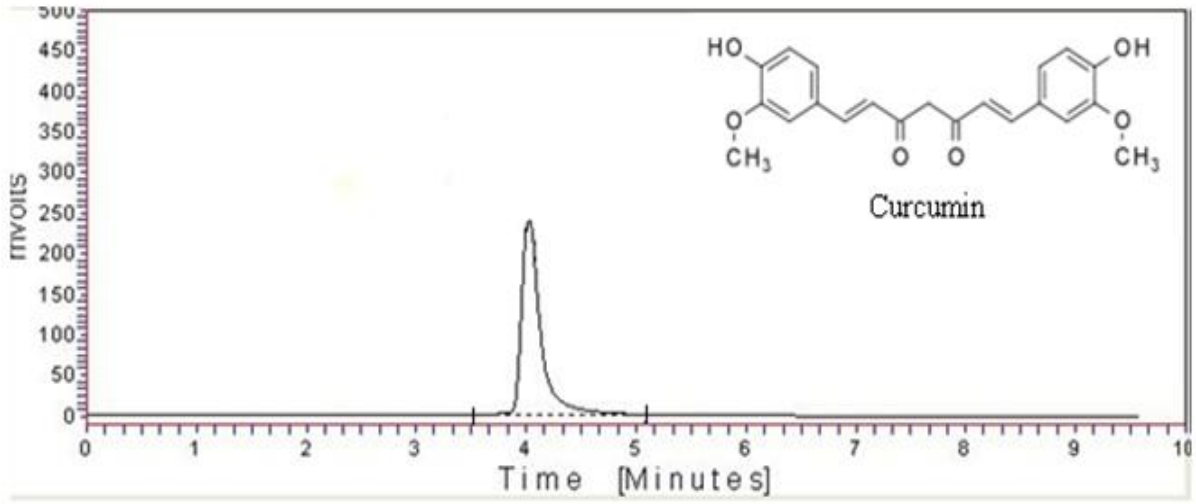

Fig. 3: HPLC spectrum of curcumin. Sample curcumin in acetonitrile, tetrahydrofuro and 2% acetic acid (50:30:20v/v) showed retention time 4.017 as compared to reported retention time 4.578 in Yadav*at el.*, 2009.

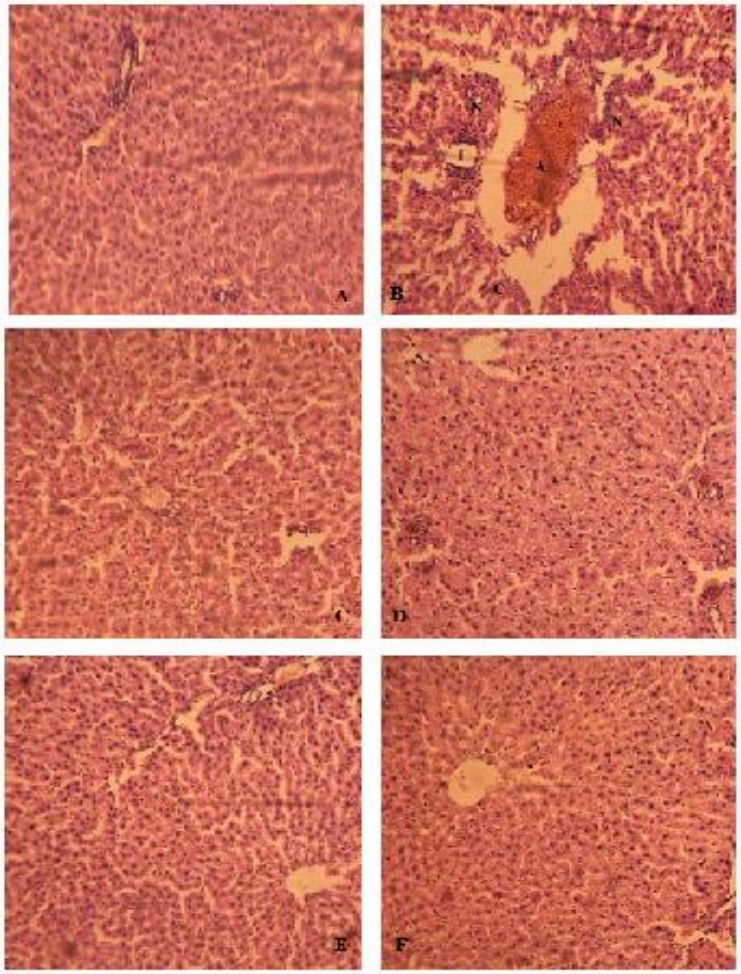

Fig. 4: Effect of picroliv and curcumin on liver histology of low calorie diet feeding and alcohol ingestion induced liver cirrhosison rats.A: Liver section of low calorie diet fed rat showing liver hepatocytes with normal architecture. B: Liver section of low calorie diet and ethanol exposed rat showing (A) abscess, (C) cirrhosis, (N) necrosis, (I) inflammatory changes, neutrophilic infiltration, damaged sinusoids, and hemorrhage. C: Liver section of low calorie diet along with ethanol exposed rat treated with silymarin (20 mg/kg) showing moderate hepatic damage with focal necrosis and cirrhosis, the absence of inflammation and absence formation.

D: Liver section of low calorie diet along with ethanol exposed rat treated with picroliv (30 mg/kg) showing moderate hepatic vein damage with hemorrhage, the presence of inflammatory neutrophilic change with few focal necrotic sites. E: Liver section of low calorie diet along with ethanol exposed rat treated with curcumin (70 mg/kg) showing normal hepatic vein and sinusoids. Hemorrhagic damage was absent from the presence of few focal necrotic damage and inflammatory sites. F: Liver section of low calorie diet along with ethanol exposed animal treated with picroliv and curcumin combination showed insignificant hepatic vein damage, neutrophilic infiltration and sinusoid dilation.

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