THERAPEUTIC EFFICACY OF *PIMPINELLA TIRUPATIENSIS* (APIACEAE) ON ACETAMINOPHEN INDUCED HEPATOTOXICITY AND OXIDATIVE STRESS IN MALE ALBINO RATS

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Summary

Pimpinella tirupatiensis (Apiaceae) is a traditional medicinal plant that is commonly used for anti-tumorogenic, anti-microbial, purgative, hypoglycemic, abortificient, analgesic, anti-septic, anti-pyretic and anti-inflammatory and improve liver conditions in India and other Asian countries. The development of hepatotoxicity induced by acetaminophen is promoted by oxidative stress. The aim of the present study was to investigate the hepatoprotective and antioxidant activities of the ethanol extract of *Pimpinella tirupatiensis* (EEPT) at two doses level of 250mg/kg & 500 mg/kg B/W on acetaminophen- induced hepatotoxicity in rats. It observed that the ethanol extract of EEPT conferred hepatoprotectivity. Biochemical and Histopathological observations confirmed the beneficial roles of ethanol extract of *Pimpinella tirupatiensis* against acetaminophen induced liver injury in rats. The activity of ethanol extract of EEPT (500 mg/kg B/W) was comparable to the standard drug silymarin (25mg/kg B/W).

Key words: Hepatoprotective, Pimpinella tirupatiensis, Acetaminophen, Silymarin Antioxidant.

Introduction

Liver diseases remain as one of the serious health problems. However there are no satisfactory liver protective drugs in allopathic medical practice for serious liver disorders. Herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes of the liver [1]. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practice as well as traditional system of medicine in India [2]. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects [3].

Pimpinella tirupatiensis (Apiaceae) locally known as 'adavikothimeera' (forest coriander) is an herbaceous medicinal plant, distributed on Tirumala Hills of Chittoor district, Andhra Pradesh, India [4]. Dried roots of *P. tirupatiensis* are administered along with few other ingredients to cure colic and rheumatic ailments in cattle [5]. The local Yanadhi tribal community uses the tuberous roots of *P. tirupatiensis* to cure severe ulcers of stomach, throat and genital organs and also as aphrodisiac [6] and as abortifacient agents [7]. Fruits are used to cure asthma and are considered as an effective remedy for 'flatulent colic' [6]. The whole plant of *P. tirupatiensis* is used to treat cough, stomach, liver problems, asthma and tooth ache [8].

However, no work has been reported on the hepatoprotective and antioxidant properties of this plant. Keeping this in view, the present study has been undertaken to investigate hepatoprotective and antioxidant activities role of the ethanol extract of *P. tirupatiensis* against acetaminophen induced liver damage in rats.

Materials and Methods

Plant material

The whole plant of *P. tirupatiensis* was collected from Tirumala Hills of Chittoor district, Andhra Pradesh, India and the plant material was taxonomically identified and authenticated by the Dr. Madhava chetty (Research Officer) botany, Andhra Pradesh. Voucher specimen (AECBT-05/2007-2008) of this plant has been retained in the Anna bioresearch foundation Arunai engineering college, Tiruvannamalai, Tamilnadu, India.

Extraction

The whole plant was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol (90% v/v) in soxhlet apparatus at 60^{o}C [9]. The solvent was completely removed by rotary vacuum evaporator. The extract was freeze dried and stored in a vacuum desiccator.

Animals

Studies were carried out using Wistar albino male rats (150-200g), obtained from Indian Veterinary Preventive medicine (IVPM), Ranipet, Tamilnadu, India. The animals were grouped and housed in polyacrylic cages (38 x 23 x10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 20C) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by poultry research station, Nandhanam, India and fresh water *ad* libitum. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the University animal ethical committee.

Drugs and Chemicals

Silymarin was purchased from Micro labs, Tamilnadu. India. Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin and total protein kits were procured from Span Diagnostics, Surat, India, and the rest of the chemicals utilized were of analytical grade and were obtained from Ranbaxy research laboratory, Hyderabad, India.

Experimental treatments

Animals were divided into five groups of six animals each. Group I treated with vehicle (distilled water) was kept as normal. Group II treated with a single dose of acetaminophen (AAP) of 750mg/kg body weight was kept as toxin control. Group III and IV were treated with ethanol extract of *P. tirupatiensis* 250 and 500 mg/kg body wt plus AAP and Group V were fed with standard drug silymarin 25mg/kg daily for seven days. The extract was administered by oral gavages 1 h before AAP administration [10].

Preparation of serum from blood

After 24 h, animals were sacrificed by chloroform anaesthesia. Blood was collected by heart puncture. The blood samples of each animal were taken and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 600×*g* for 15 min and analyzed for various biochemical parameters including serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT) [11] alkaline phosphatase (ALP) , [12] bilirubin [13] and total protein [14].

Preparation of liver homogenate

Hepatic tissues were homogenized in KCl [10 mM] phosphate buffer (1.15%) with ethylene-diamine tetra acetic acid (EDTA; pH 7.4) and centrifuged at 12,000×g for 60 min. The supernatant was used for assay of the marker enzymes (glutathione peroxidase, glutathione-s-transferase, superoxide dismutase and catalase), reduced glutathione, thiobarbituric acid reactive substances (TBARS) content, and protein estimation.

Biochemical estimation of markers of oxidative stress

MDA content was Measured according to the earlier method reported [15]. SOD activity was determined according to previous report [16]. CAT activity was determined from the rate of decomposition of H_2O_2 by the reported method [7]. GPX activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN3 [18]. Glutathione reductase activity was assayed according to previous reports [19-20] . Protein content in the tissue was determined by earlier method reported [21], using bovine serum albumin (BSA) as the standard.

Histopathological study

Liver specimens from all the experimental groups were fixed in 10% buffered formalin and were processed for paraffin sectioning. Sections of about 5 µm thickness were stained with haematoxylin and eosin to study the general structure of the liver.

Statistical analysis

The obtained results were analyzed for statistical significance using one way ANOVA followed by Dunnet test statistical software for comparison with control group and acetaminophen treated group. $P < 0.05$ was considered as significant.

Results

The effect of *Pimpinella tirupatiensis* on serum marker enzymes is presented in fig 1, 2 & 4. The serum levels of GOT, GPT, ALP and total bilirubin were markedly significantly ($p< 0.01$) elevated and that of protein levels significantly ($p< 0.01$) decreased in acetaminophen treated animals, indicating liver damage. Administration of ethanol extract of *Pimpinella tirupatiensis* at the doses of 250 and 500 mg/kg remarkably significantly ($p< 0.05$; $p< 0.01$) decreased the levels of GOT, GPT and ALP along with increased the total protein content, which is indicated that prevented the hepatotoxicity induced by acetaminophen.

Analysis of MDA levels by thiobarbituric acid reaction showed a significant ($P<0.01$) increase in the acetaminophen treated rats. Treatment with EEPT (250 mg/kg & 500 mg/kg) significantly ($P<0.01$; $P<0.01$) prevented the increase in MDA level which was brought to near normal (fig 1). Acetaminophen treatment caused a significant ($P<0.01$) decrease in the level of SOD, catalase, GPX and GST in liver tissue when compared with control group. The treatment of EEPT at the doses of 250 and 500 mg/kg resulted in a significant ($P<0.05$; $P<0.01$) increase of SOD, catalase, GPX and GST when compared to Group I (Fig 2 & 3). The standard drug, silymarin treated animals also showed a significant ($P<0.01$) increase in antioxidant enzymes levels compared to Group I. Morphological observations showed an increased size and enlargement of the liver in acetaminophen treated groups. These changes were reversed by treatment with silymarin and also EEPT at the two different doses tested groups.

Histopathological profile of the normal animal showed normal hepatocytes with well preserved cytoplasm and there was no sign of inflammation, which has been illustrated in Fig 5 (a). The acetaminophen treated animals showed severe centrilobular necrosis and fatty infiltration (Fig 5 b). Treatment with different doses of ethanol extract of EEPT and silymarin produced mild degenerative changes and absence of centrilobular necrosis when compared with control [Fig 5 (c), 5 (d) & 5 (e)]. All these results indicate a hepatoprotective potential by the ethanol extract of EEPT.

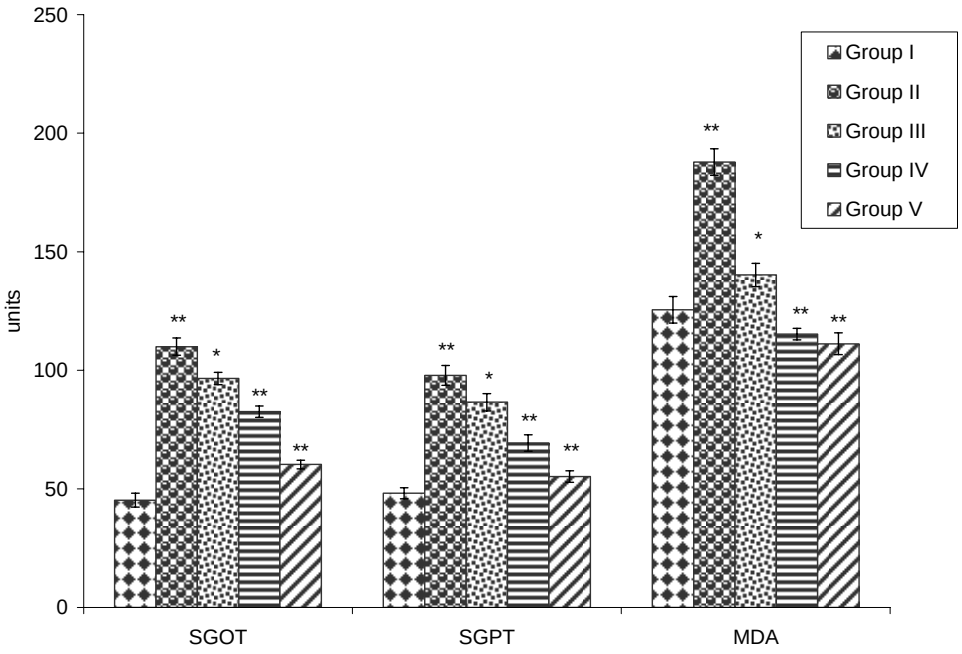

Fig.1. Effect of ethanolic extract of *Pimpinella tirupatiensis* and silymarin (standard drug, (25 mg/kg)) on serum levels of SGOT (IU/L), SGPT (IU/L) and MDA (nM/mg of protein) [Lipid peroxidation (LPO)] level of hepatic tissue during acetaminophen treated hepatotoxicity and oxidative stress in rats. Values are mean ± S.D. (n = 6). **p < 0.01, *p < 0.05, respectively.

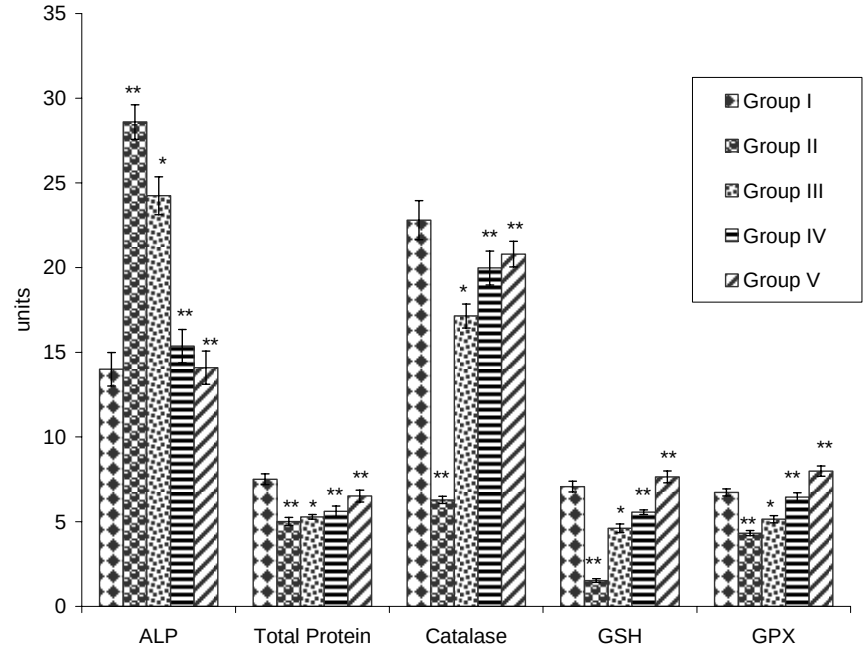

Fig.2. Effect of ethanolic extract of *Pimpinella tirupatiensis* and silymarin (standard drug, (25 mg/kg)) on serum levels of alkaline phosphotase (ALP) (IU/L) & total protein and hepatic levels of CAT (U/mg protein), GSH (U/mg protein) and GPX (micrograms of glutathione utilized/min/mg protein) during acetaminophen treated hepatotoxicity and oxidative stress in rats. Values are mean ± S.D. (n = 6). **p < 0.01, *p < 0.05, respectively.

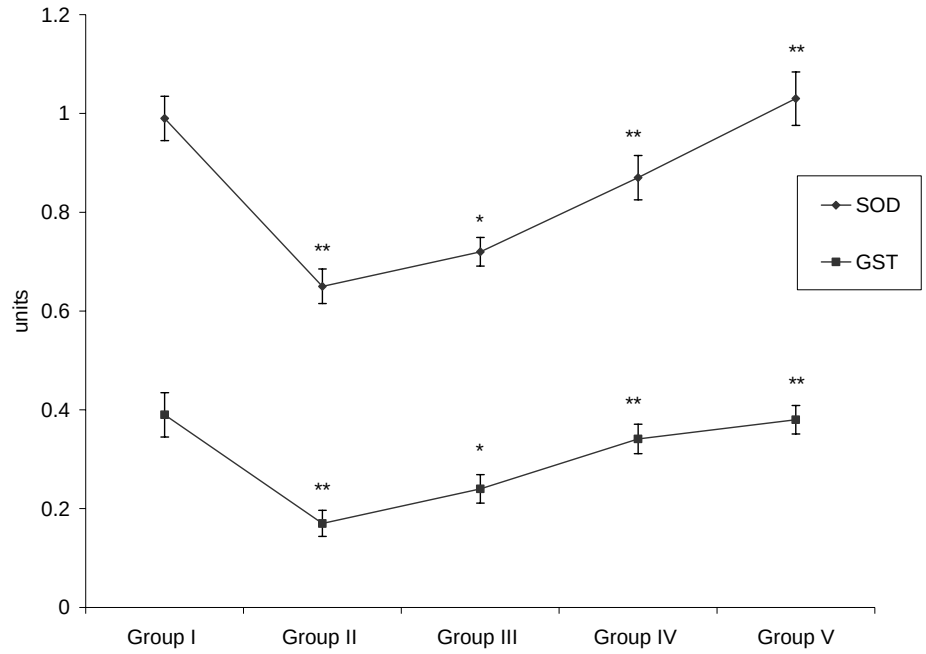

Fig.3. Effect of ethanolic extract of Pimpinella tirupatiensis and silymarin (standard drug, (25 mg/kg) on hepatic levels of SOD(units of activity/mg protein) & GST (Units/mg protein) during acetaminophen treated hepatotoxicity and oxidative stress in rats. Values are mean ± S.D. (n = 6). **p < 0.01, *p < 0.05, respectively.

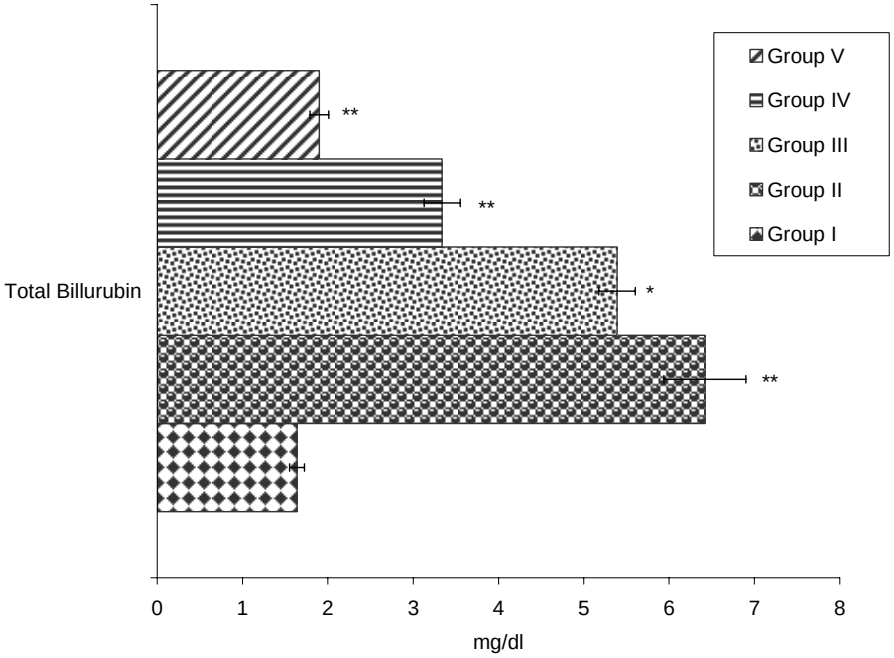

Fig.4. Effect of ethanolic extract of *Pimpinella tirupatiensis* and silymarin (standard drug, (25 mg/kg)) on serum levels of total bilurubin (mg/dl) during acetaminophen treated hepatotoxicity and oxidative stress in rats. Values are mean ± S.D. (n = 6). **p < 0.01, *p < 0.05, respectively.

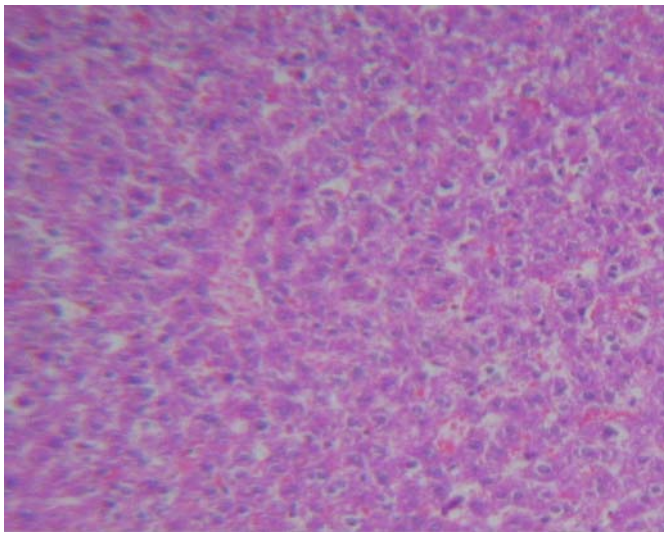

Fig. 5. (a). Normal photomicrograph of liver tissue of control rat showing normal hepatic cells with central vein and sinusoidal dilation. (H and E 100X).

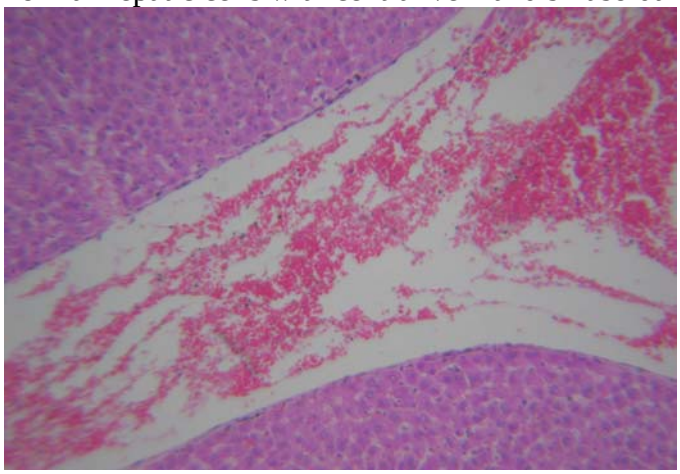

Fig.5. (b). Liver section of rat showing disarrangement and degeneration of normal hepatic cells with centrilobular necrosis extending to mid zone and sinusoidal hemorrhages and dilation. (H and E 100X).

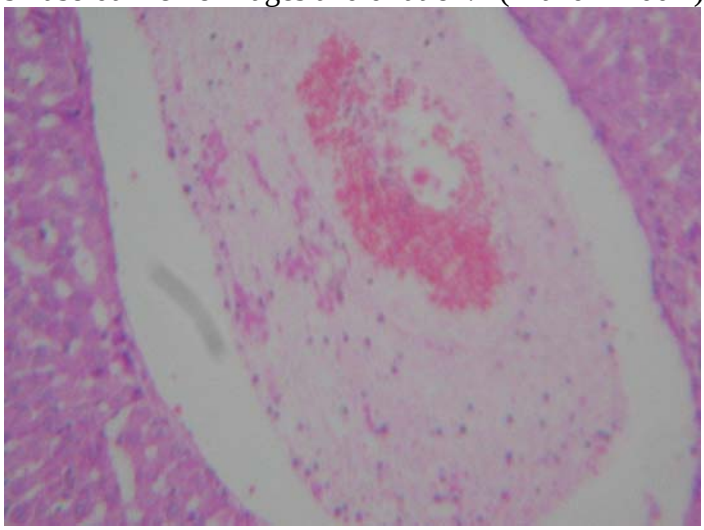

Fig. 5. (c). Histology of liver from rat which received *Pimpinella tirupatiensis* ethanolic extract at 250 mg/kg.(Group III) showing mild degenerative changes and absence of centrilobular necrosis. (H and E 100X).

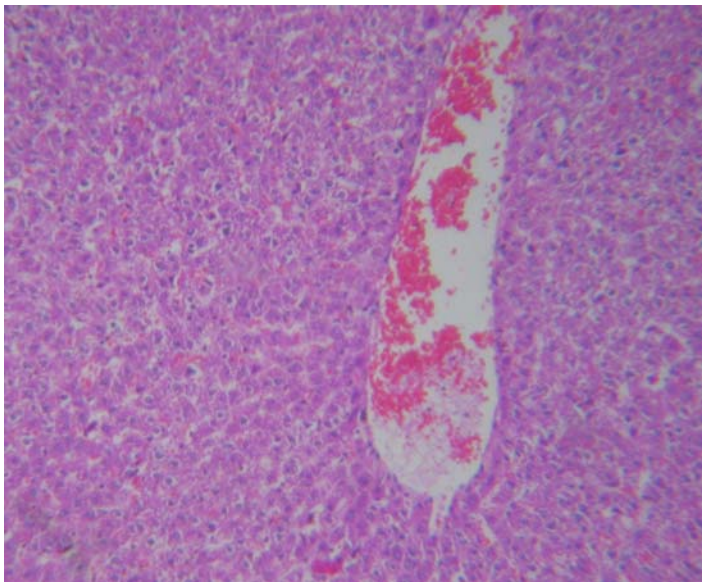

Fig. 5. (d). Histology of liver from rat which received *Pimpinella tirupatiensis* ethanolic extract at 500mg/kg(Group IV) showing normal hepatocytes with mild inflammation .(H and E 100X).

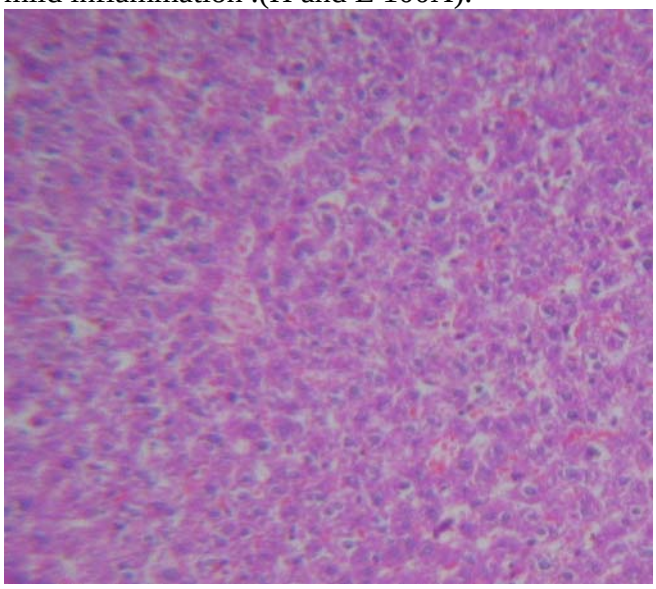

Fig.5.(e). Liver section of Rat treated with silymarin at 25 mg/kg showed less vacuole formation reduced sinusoidal dilation, less disarrangements and degeneration of hepatocytes. (H and E 100X).

Discussion

Acetaminophen (APAP), also known as paracetamol is a commonly used analgesic and antipyretic drug. The drug is safe at therapeutic levels, but an acute APAP overdose can lead to potentially fatal hepatic and renal necrosis in humans and experimental animals [22- 25]. It has been suggested that acetaminophen (APAP)- induced hepatic injury is produced by the cytochrome P-450 generated reactive metabolite, N-acetyl-pbenzoquinoneimine (NAPQI), which is normally conjugated with glutathione and excreted in urine. In overdose situations, however, glutathione levels are exhausted and NAPQI can directly modify susceptible protein residues in

what is widely believed to be the first step in a cascade of biochemical events leading to hepatocyte death [26-31]. The major finding observed in acetaminophen intoxication in humans and animals is acute centrolobular necrosis [32-33].

In the present study, AAP induced rat liver necrosis observed in the histopathological findings (Fig 5(b)). Increase in the serum GOT level is considered to be a significant indicator of AAP -induced acute liver damage [34 -35]. In the present study, when compared to the control group, the increase($P<0.01$) detected in the serum GOT and GPT levels of rat which were given AAP , demonstrates APAP -induced acute liver damage to have developed, increased ALT and AST levels are considered to be specific to liver damage [36 -37]. Increased levels of serum ALT and AST in rat treated with acetaminophen indicate a deterioration of the hepatic functions due to the toxic effects of the drug. The increase in collagen contents implicates oxidative liver injury while the histopathological data of the tissues confirms the acetaminophen-induced organ damage [38].

There was a significant ($P<0.01$) restoration of these enzyme levels on administration of the EEPT in a dose dependent manner and also by silymarin at a dose of 25 mg/kg. The reversal of increased serum enzymes in acetaminophen induced liver damage by the EEPT may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [39-40]. Effective control of ALP, bilirubin and total protein levels points towards an early improvement in the secretary mechanism of the hepatic cells, as well as repair of hepatic tissue damage caused by AAP. This indicates the anti-lipid per oxidation and/or adaptive nature of the systems as brought about by plant extract against the damaging effects of free radical produced by AAP.

Decrease in enzyme activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in live injury [41]. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. In EEPT causes a significant increase in hepatic SOD activity and thus reduces reactive free radical induced oxidative damage to liver.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals [42]. Therefore reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide. A higher dose (500 mg/kg) of EEPT and silymarin significantly increased the level of CAT.

Both reductions of GPX &GSH activity AAP-treated rats as observed in this study indicate the damage to the hepatic cells. Administration of EEPT promoted the reactivation of hepatic glutathione reductase enzyme in AAP-treated rats. The restoration of GSH level after the administration of ethanol extract *Pimpinella tirupatiensis* to such AAP treated rats due to the protective effect.

The increase in MDA level in liver induced by acetaminophen suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with EEPT significantly reverses these changes. Hence it is likely that the mechanism of hepatoprotection of EEPT is due to its antioxidant effect.

Severe centrilobular necrosis and fatty infiltration in in hepatocytes was produced by acetaminophen. Treatment with different doses of ethanolic extract of *Pimpinella tirupatiensis* produced only mild degenerative changes and absence of centrilobular necrosis, indicating EEPT treatment significantly recurred these signs of inflammation and necrosis, which indicated that EEPT treatment conferred hepatoprotectivity.

In conclusion, ethanol extract of *Pimpinella tirupatiensis* significantly protects against liver injuries as well as oxidative stress, resulting in improved serum biochemical parameters such as SGOT, SGPT and SALP. The reduced levels of parameters of SOD, CAT, GSH, GPX, and GST in acetaminophen-treated rats were significantly increased by treatment with ethanol extract of *Pimpinella tirupatiensis*. Further studies to characterize the active principles and to elucidate the mechanism are in progress.

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