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Research Article

HEPATOPROTECTIVE ACTIVITY OF VARIOUS EXTRACTS OF *CADABA FRUTICOSA* AGAINST PARACETAMOL-INDUCED LIVER INJURY IN RATS

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ABSTRACT

Herbal medicine has long been used for the prevention and treatment of a wide range of medical conditions, as well as for general health enhancement. Liver injury is one of the major health problems in human which sometimes may lead to even death. Herbal products may be the most excellent source of remedies for the treatment of liver diseases. Thus the innovation of a potential therapeutic agent for the safety of the liver from the hepatotoxins will provide a useful way for the prevention of these liver-related illnesses. The aim of the present study is to evaluate the hepatoprotective effect of Cadaba Fruticosa extract against paracetamol-induced liver damage in male Wistar rats. Administration with petroleum ether and chloroform extract of Cadaba fruticosa extracts for 28 days significantly reduced the impact of Paracetamol toxicity on the serum markers of liver damage, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase and total bilirubin. Liv.52 was used as a positive control. The effects of the drug were decided by alteration in serum marker ALT, AST, ALP, Protein and bilirubin levels. Both the extract shows better the hepatoprotective activity at 400 mg/kg and at lower dose shows marked activity. The histopathological studies in the liver of rats also supported that Cadaba Fruticosa extract was effectively reduced the hepatotoxicity of paracetamol. Thus, the results suggest that Cadaba Fruticosa extract acts as a potent hepatoprotective agent against paracetamol-induced hepatotoxicity in rats.

KEYWORDS: *Cadaba Fruticosa, Hepatoprotective, Paracetamol.*

INTRODUCTION

In prehistoric Indian novel, it is mentioned that all plants in this world it is useful for human beings, animals and other plants. The liver as a very important organ in the body is mostly responsible for the metabolism of endogenous and exogenous agents. It plays an important role in drug elimination and detoxification [1]. Although viruses are the main cause of liver diseases, environmental pollutants, xenobiotics, hepatotoxins, excessive drug therapy and chronic alcohol ingestions can also cause severe liver injury [2]. Hepatotoxicity is defined as an injury to the liver that is associated with impaired liver function caused by exposure to a drug or another non-infectious agent [3]. However, chemical toxins (including Paracetamol, carbon tetrachloride (ccl_4), galactosamine and thioacetamide) are often used as the chemical agents causing experimental hepatocyte injury in both in vivo and in vitro conditions [4]. Jaundice and hepatitis are two major hepatic disorders that relate to a high death rate [5]. At present only a few hepatoprotective drugs and those from natural sources are available for the treatment of liver disorders [6]. More than 900 drugs have been concerned with causing liver injury and it is the most familiar reason for a drug to be quiet from the market [7]. Every year 5% of liver injury patients admitted in hospital and 50% patients of acute liver failures [8].

Cadaba fruticosa (L.) Druce is most suitable for the viable exploitation and development of limited source based herbal drugs. It is popularly known as in English 'Capper Brush' is locally recognized under different names like 'vuldhi' in Tamil. This plant can be flowering and fruiting from November to April. Leaf aqueous extract exposed terpenoids, flavones, proteins, furans; anthraquinones and sugars are present and alcoholic extract posses steroids, alkaloids, gums and saponins [9]. This Leaf juice is used as a treatment for dysentery, stimulant, purgative, fever, cough and lungs problem [10]. Leaves are reported to possess antimicrobial activity [11]; anti-pyretic activity [12], anti-diabetic activity. A decoction of the leaves used to treat anthelmintic, dyspepsia in children, amenorrhea, dysmenorrhea, treatment of rheumatic pain deobstruent, emmenagogue and aperients and liver diseases [13].

Acetaminophen, a widely used analgesic and antipyretic drug commonly used for pain and fever relief. It is commonly considered as a "safe drug" when taking within the suggested therapeutic dose. But in higher doses, it causes hepatotoxicity in humans and experimental models. As the ingested dose of acetaminophen is increased, hepatic

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glutathione stores become progressively depleted. Consequently, glutathione available for scavenging oxygen radicals is brought down, resulting in an increase in reactive oxygen. This will be thus associated with a concurrent increase in lipid peroxidation and other hydroperoxides. When the formation of N-acetyl-p-benzoquinone imine (NAPQI) is of sufficient magnitude, glutathione stores will fall below a critical level that is no longer adequate to sustain detoxification of NAPQI (N-acetyl-p-benzoquinone imine). At this point, the disruption of cellular structure and function occurs due to the covalent binding of NAPQI to cellular macromolecules such as proteins and lipids thereby leading to hepatic necrosis [14]. The main purpose of this study was to assess the hepatoprotective effect of *Cadaba fruticosa* (L.) Druce in paracetamol-induced hepatotoxicity.

MATERIALS AND METHODS

Chemicals:

Petroleum ether was obtained from Sigma – alrich chemicals pvt ltd., Bangalore. Methanol obtained from Molychem, Mumbai. Chloroform was obtained from Himedia laboratories pvt, ltd.mumbai. Assay kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin kit were purchased from Agappe diagnostics ltd., ernakulam, kerala. All other chemicals used in this study were of analytical grade.

1. Preparation of the plant extraction:

i. Petroleum ether extract of leaves of Cadaba fruticosa: The dried coarse powder of leaves of Cadaba fruticosa was extracted with one litre of petroleum ether (60 - 80°C) by continuous percolation method using soxhlet apparatus, after 74 hours the extraction was completed then petroleum ether was taken and the solvent was redistilled. A dark Greenish yellow colour was obtained [15].

ii. Chloroform extract of leaves of Cadaba fruticosa: The march left after extraction, was dried and subsequently extracted with 1 litre of chloroform by continuous percolation method. After 74 hours, the extraction was completed, it was filtered and the solvent was removed by distillation under reduced pressure. A dark green coloured residue was stored in a desiccator and the marc was dried for further extraction [16].

2. Preliminary Phytochemical Analysis:

The various extracts of *Cadaba fruticosa* obtained were subjected to qualitative analysis to test the presence of various phytochemical constituents like alkaloids, carbohydrates, glycosides, flavonoids, steroids, aminoacid, phenols, proteins, tannins etc [17-19].

3. Acute Toxicity Study:

Acute oral toxicity test was carried out as per the Organization for Economic Co-operation and Development (OECD-423) guidelines for testing of chemicals. Six randomly selected male rats were used for the acute toxicity study of each extracts [20]. For a sighting study, a single rat was fasted overnight and administered with a dose of 4000 mg/kg of each extract orally by gavage. Then, the rats also fasted for 4 h with no access to food after extract administration. Immediately after dosing, the animals were observed continuously for the first 4h with 30 min intervals and until 24 h for any behavioral changes (paw-licking, motor activity, tremors, convulsions, posture, spasticity, opisthotonicity, ataxia, sensations, pilo-erection, ptosis, lacrimation, exopthalmos, salivation, diarrhoea, writhing, skin colour, respiratory rate and mortality) and sign of toxicity [21]. Since no death was observed within 24 h, additional two animals were added for each extracts and administered the same dose. The animals were observed continuously for 4 h with 30 min interval and then for 14 consecutive days with an interval of 24 hrs.

4. Pharmacological Evaluation:

Animals used:

Adult male albino rats (150 - 200g) were used in this study. Animals are placed in clean, sterile, polypropylene cages and fed with commercial pellet rat chow (M/S Hindustan lever limited, Bangalore, India) and water ad libitum. The study was approved by the Institutional Ethical Committee, which follows the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA).

Experimental design for hepatoprotective activity:

The rats were divided into seven groups each containing four rats.

Group I: Control group of animals received Normal saline

Group II: treated with silymarin (100 mg/kg)

Group III: Paracetamol control which received paracetamol (3 g/kg) on the 7thday

Group IV: treated with Petroleum ether extract of leaves of *Cadaba fruticosa* (200 mg/kg)

Group V: treated with Petroleum ether extract of leaves of *Cadaba fruticosa* (400 mg/kg)

Group VI: treated with Chloroform extract of leaves of *Cadaba fruticosa* (200 mg/kg)

Group VII: treated with Chloroform extract of leaves of *Cadaba fruticosa* (400 mg/kg)

The treatment was continued for seven days. On 8th day a single dose of paracetamol (3000 mg/kg) suspension was given to Groups II-VII. After 48 hrs of paracetamol administration, blood was collected from all the groups of rats via retro-orbital plexus route. The blood samples were collected in glass test tubes and allowed to coagulate for 30 min. Serum was separated by centrifugation at 3000 rpm for 20min and used for evaluating the biochemical parameters such as SGOT, SGPT, ALP and total bilirubin.

5. Histopathology study of liver:

A portion of liver tissue in each group was fixed in 10% formalin and proceded for histopathological studies for evaluation of hepatocytes cells, sinusoidal congestion and mononuclear inflammatory cells of different groups.

6. Statistical analysis:

The results are represented as mean + S.E.M. Students't-test is used for statistical analysis of blood serum parameters and for statistical analysis of liver enzymes.

RESULTS

Histopathological studies of the liver in paracetamol-induced hepatotoxicity:

The histopathological evaluation of paracetamol toxicity in all the groups was examined and shown in the figure.

The description is as follows, the section of rat liver treated with the vehicle control group shows liver parenchyma with intact architecture which is the normal appearance. Section of paracetamol-induced toxic control group show trinities with scattered inflammatory infiltration within the parenchyma which is due to the toxicity. Section of silymarin treated group shows liver parenchyma with intact architecture. Section of the liver in the test drugs pet ether and chloroform extract-treated groups shows the intact architecture, few regenerative hepatocytes and sinusoidal congestion which is similar to silymarin treated group (Fig. 1 to 7).

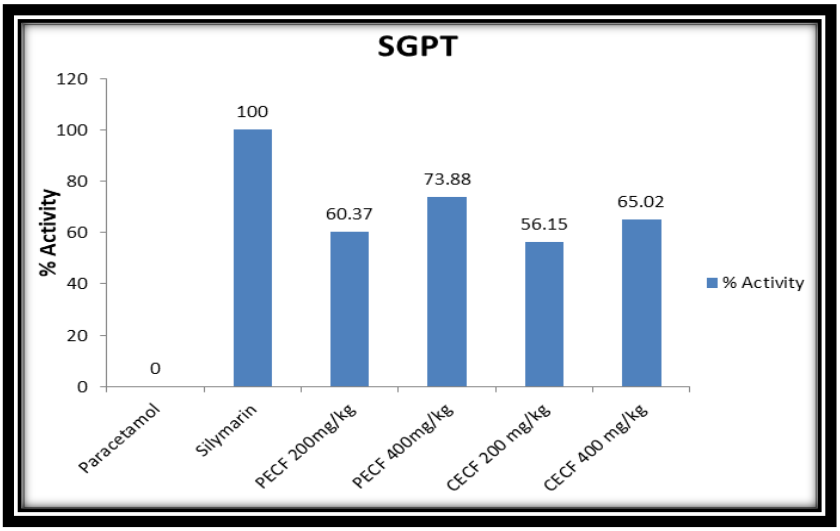
Table No. 1: Phytochemical screening of various extracts of leaves *Cadaba fruticosa* leaves

S. No.	Phytochemical Constituent	Extracts	
		Petroleum ether extract	Chloroform extract
1.	Carbohydrates	-	-
2.	Glycosides	-	+
3.	Proteins & Aminoacids	-	-
4.	Tannins & phenolics	-	-
5.	Terpenoids	+	+
6.	Flavonoids	+	+
7.	Phytosterols	+	+
8.	Fixed oils &Fats	+	+
9.	Alkaloids	-	-
10.	Saponins	+	+
11.	Gums & Mucilages	-	-

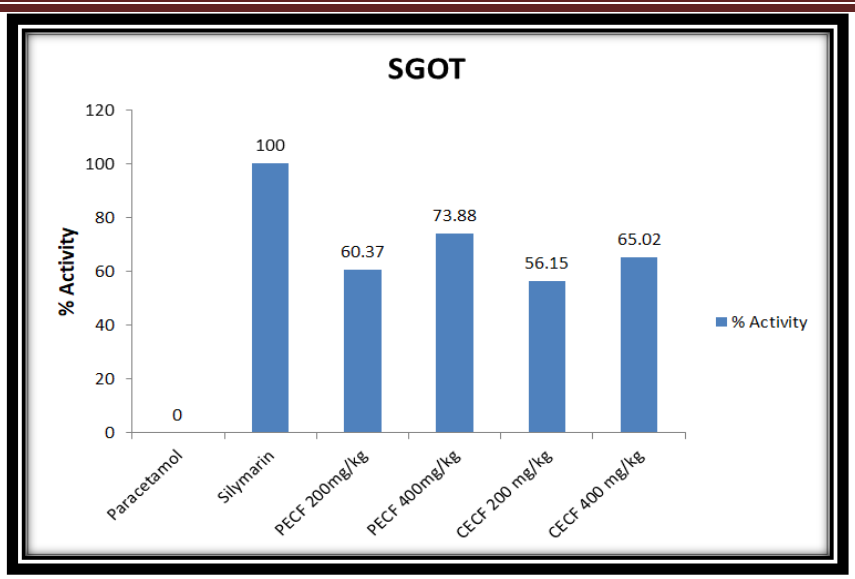
Table No. 2: Effect of extracts of *Cadaba fruticosa* leaves on serum enzyme SGPT and SGOT on Paracetamol – intoxicated rats

Treatment	Dose (mg/kg)	SGPT Level Mean ± SEM	% Activity	SGOT Level Mean ± SEM	% Activity
Control	Tween 80	270.98 ± 0.742	-	306.82 ± 0.55	-
Paracetamol	3000	793.91± 0.95	-	824.96 ± 1.05	-
Standard (Silymarin)	200	322.85 ± 1.4***	100%	328.26 ± 1.1***	100%
PECF	200	506.02 ± 0.92**	63.8%	543.69 ± 0.92**	60.37%
PECF	400	384.46 ± 1.58**	83.97%	444.28 ± 0.71**	73.88%
CECF	200	562.12 ±1.45**	57.43%	584.52 ±0.86**	56.15%
CECF	400	511.97 ± 1.07**	63.06%	504.79 ±1.4**	65.02%

Results are expressed as means ± SEM, n=4 **p<0.01, ***p<0.001 when compared to normal control group



Graph. 1: Percentage reduced in the elevated level of Serum SGPT

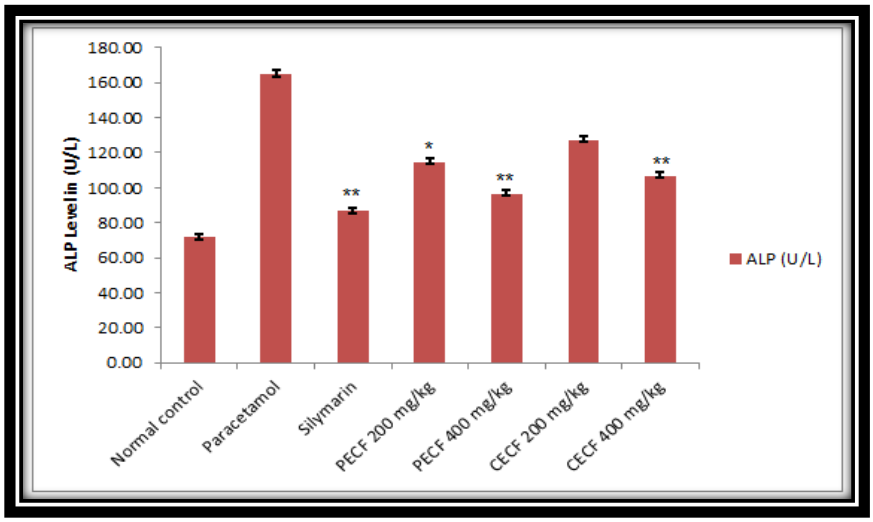


Graph. 2: Percentage reduced in the elevated level of Serum SGOT

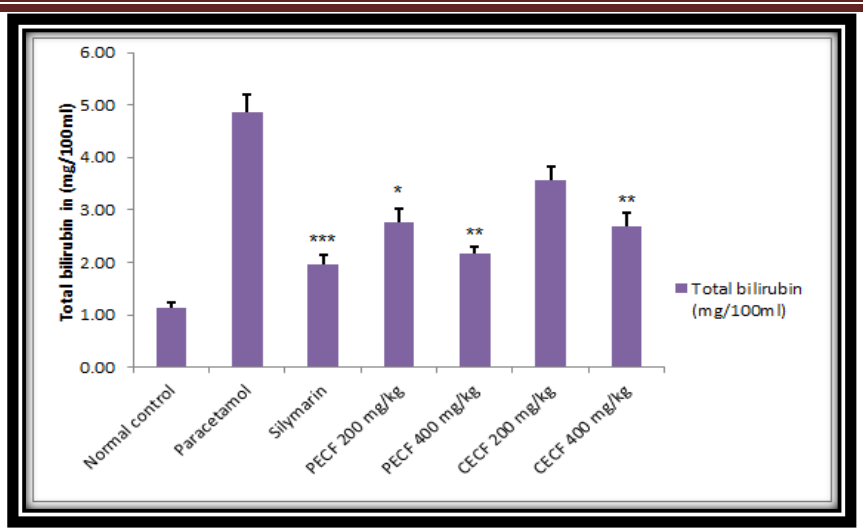
Table No. 3: Effect of pet ether and chloroform extract of *C.fruticosa* on ALP and Total bilirubin level in Paracetamol intoxicated rat

Treatment	ALP (U/L)	Total Bilirubin (mg/100ml)
Normal Control	71.73 ± 1.82	1.12 ± 0.11
Paracetamol	164.61 ± 2.58	4.86 ± 0.34
Silymarin	86.62 ± 1.84**	1.95 ± 0.18***
PECF 200	114.75 ± 2.29*	2.76 ± 0.25*
PECF 400	96.51 ± 1.88**	2.18 ± 0.13**
CECF 200	127.38 ± 2.32	3.56 ± 0.26
CECF 400	106.5 ± 2.10**	2.68 ± 0.27**

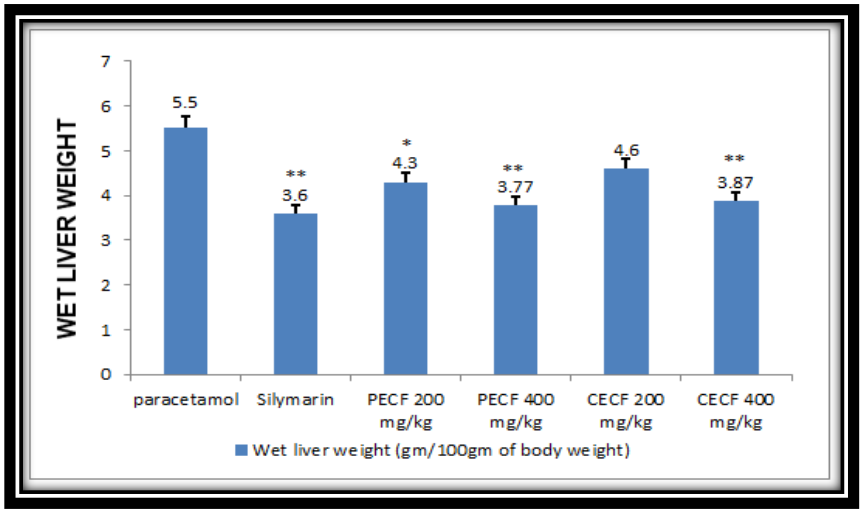
Results was expressed in Mean ± SEM *p<0.05, **p<0.01, ***p<0.001 as comparison with the control group



Graph. 3: Effect of extracts of Cadba fruticosa on ALP enzyme on Paracetamol intoxicated rats



Graph. 4: Effect of extracts of Cadaba fruticosa on Total Bilirubin on Paracetamol intoxicated rats



Graph. 5: Effect of extract of C.fruticosa on wet liver on paracetamol intoxicated rats

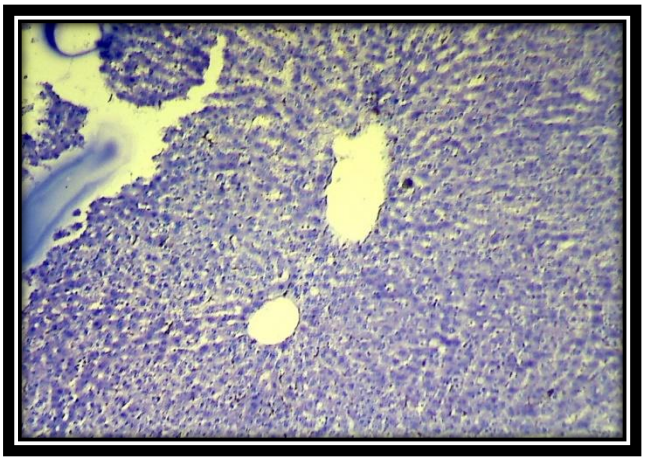


Fig. 1: Normal control group, showing normal hepatocytes

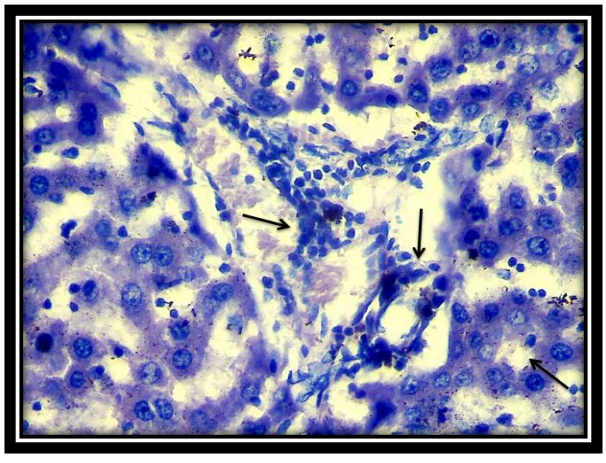


Fig. 2: Paracetamol treated animal group shows hepatic damage, sinusoidal congestion of liver and kupffer cell hyperplasia

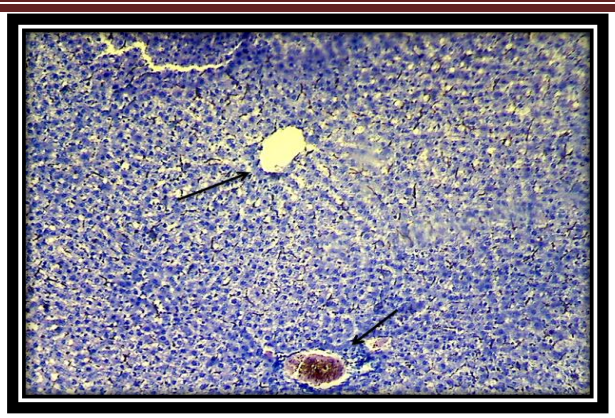


Fig. 3: Hepatocytes of Standard (Silymarin 200mg/kg) treated group

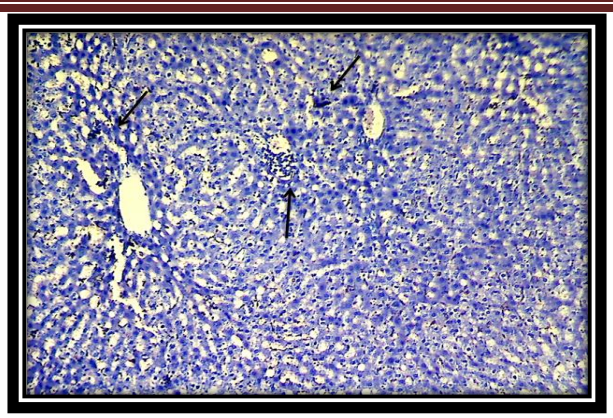


Fig. 4: Pet ether 200 mg/kg extract treated group shows show regenerative hepatocytes and Triaditis

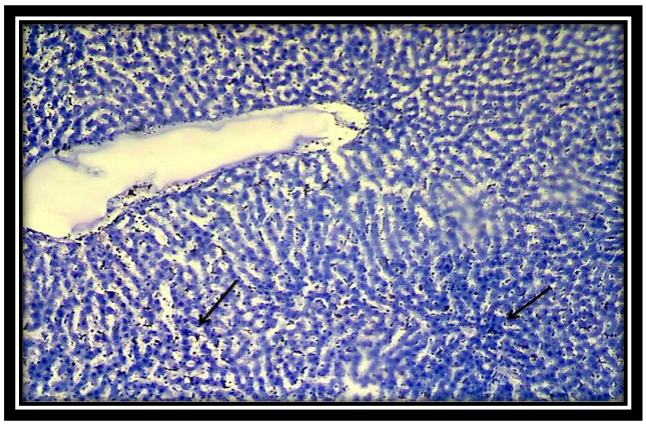


Fig. 5: Pet ether 400mg/kg treated group shows few regenerative hepatocytes and sinusoidal congestion

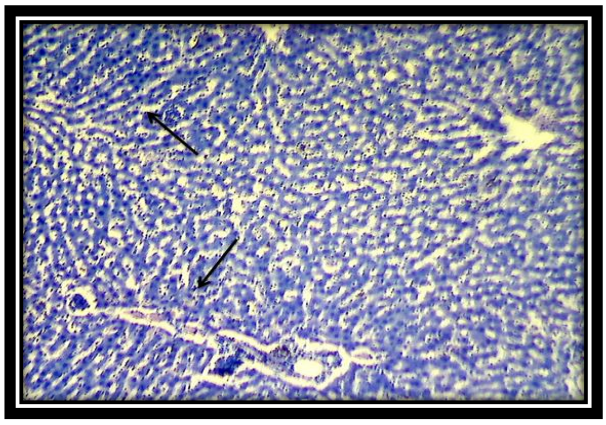


Fig. 6: Chloroform extract 200mg/kg treated group shows that regenerative hepatocytes, Triaditis and sinusoidal collections of lymphocytes

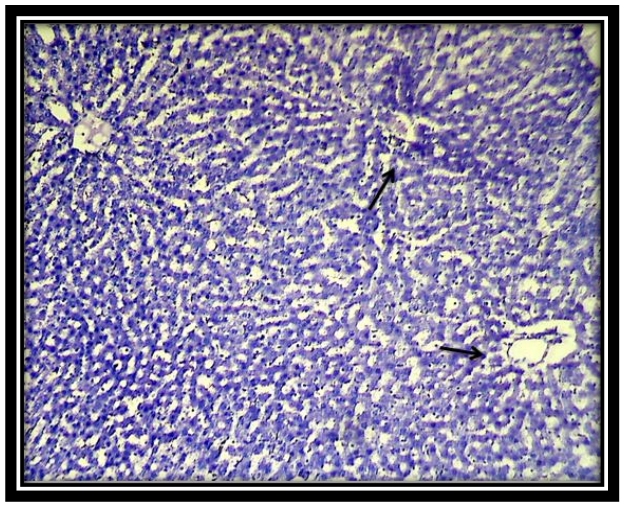


Fig. 7: Chloroform extract 400 mg/kg treated group shows that regenerative hepatocytes, sinusoidal congestion and scattered mononuclear inflammatory cells which is similar to silymarin treated group

DISCUSSION

Paracetamol is one of a commonly used antipyretic agent; which is safe up to the level of preferred dose. When this medication is taken in a higher dose (toxic dose) which cause severe damage to the liver. Because major metabolism of paracetamol takes place in the liver, with help hepatic enzymes mainly by Cytochrome P-450. Mostly in safe dose liver detoxify the drug by converting them to sulfate and glucuronide conjugation which are inactive. Then a small amount of drug which is oxidized by cytochrome P-450 hepatic enzyme which converts into a highly reactive intermediate product known as N-acetyl-P-benzoquinone imine (NAPQI) which is deactivated

by glutathione. During the toxic dose level, where their high concentration of drug which causes inactivate the sulfate and glucuronide pathways which lead to shut down the action of Cytochrome P-450 activity. The excess amount of glutathione is excreted from the liver which leads to glutathione depletion which causes liver damage. Because glutathione is important for the viability of the liver and promoting the regeneration of liver cells. [69] Evidence for the paracetamol inducing toxicity was reported earlier.

When liver damaged by any cause, it can be identified by the analysing the increased level of serum biochemical enzymes such as SGOT, SGPT, ALP and total bilirubin. In this study, the liver damage is induced by the intoxication of animals with paracetamol (3000 mg/kg). Likewise by analysing the blood sample for estimating the level of serum biochemical after the 48 hours of paracetamol intoxication shows marked increase in the serum enzymes level in case of paracetamol toxic group and this elevated level of serum enzymes was significantly recovered in the standard group and in the petroleum ether and chloroform extract-treated group shows somewhat near towards the normal level was observed. Both the extract shows better the hepatoprotective activity at 400 mg/kg and at lower dose shows marked activity.

In the Wet liver weight assessment paracetamol control group has increased liver weight. Then the P 0.05> significant reduction in the weight of the liver is observed in both the pet ether and chloroform extract at 400 mg/kg.

Furthur Histopathological liver section of paracetamol treated animal show sinusoidal collection and kupffer cell hyperplasia. In the case of petroleum ether and chloroform extract of *C. fruticosa* shows a decrease in severity of liver damage and shows regenerative cells which further indicates that the supplement of Plant extract of *cadaba fruticosa* shows hepatoprotective action.

CONCLUSION

Generally, research was going in the herbal plants to find a solution to tackle various diseases. Though herbal medicines are effective in the treatment of various ailments very often these drugs are unscientifically exploited or improperly used. Therefore, these herbal drugs deserve detailed studies in the light of modern medicine. In spite of synthetic drugs, herbal drugs have their place in therapy. Their effectiveness, low-cost and comparative freedom from serious toxic effects makes these medicines not only popular but also an acceptable mode of treating diseases even in modern times.

Cadaba fruticosa one of the herbal plant which is used in traditional medicine. This study reveals the phytoconstituents of leaves extracts of *Cadaba fruticosa* was identified and the Pharmacological evaluation hepatoprotective activity against paracetamol-induced hepatotoxicity was done. Then *in – vitro* anthelmintic activity was done on Indian earthworm models. In conclusion, the hepatoprotective effect of petroleum ether and chloroform extract of leaves *Cadaba fruticosa* was confirmed by the following measures; such as level of SGPT, SGOT, ALP and total bilirubin observed in paracetamol-induced hepatotoxicity. Finally, both the extract of *Cadaba fruticosa* shown significant hepatoprotective. Thus they effectively overcome the toxic mechanism of paracetamol. This proves the hepatoprotective potential of leaves extract of *Cadaba fruticosa*

In support of this study, histopathological results also show the significant activity of the plant extract. In toxicant treated animals there will be severe disturbances in the cytoarchitecture of the liver. But in the petroleum ether and chloroform extract of *Cadaba fruticosa* shows regeneration of hepatocytes was observed, which confirms the hepatoprotective activity and supports the traditional application of the same under the light of modern science.

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