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HEPATOPROTECTIVE ACTIVITY OF AQUEOUS BARK EXTRACT OF ALSTONIA SCHOLARIS IN PARACETAMOL AND ETHANOL INDUCED LIVER DAMAGE IN ALBINO WISTAR RATS

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ABSTRACT

The *In vivo* hepatoprotective effect of aqueous extract of Alstonia scholaris (AEAS) bark was evaluated in male rats against ethanol and paracetamol induced liver damage in preventive and curative models. Liver injury was induced by 40% ethanol administration (3.7 g/kg bw, orally) for 28 days and in the other case hepatic damage was induced by the administration of Paracetamol at a dose of 2g/kg bw for 11 days. In two different sets of experiments, the AEAS extracts (100, 200 and 400 mg/kg bw) and silymarin (100 mg/kg bw) were administered orally in preventive and curative models. Ethanol administration as well as Paracetamol administration caused severe hepatic damage in rats as evidenced by elevated serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin levels. The AEAS and silymarin administration prevented the toxic effect of ethanol and Paracetamol on the above serum parameters in both preventive and curative models. The present study concludes that aqueous extract of Alstonia scholaris bark showed significant hepatoprotective activity against ethanol induced hepatotoxicity as well as Paracetamol induced hepatotoxicity.

KEY WORDS: Alstonia scholaris; Ethanol; Paracetamol; Hepatoprotective; Silymarin

1. INTRODUCTION

Liver is the largest glandular organ of the body which plays a vital role in metabolizing carbohydrates, lipids, proteins and detoxifying xenobiotics and drugs. Thus the liver is proned to injury due to the chronic exposure to drugs, environmental toxicants and other

xenobiotics [1]. Today, alcohol abuse is one of the major health problems worldwide. There is a close relationship between ethanol intake and alcoholic liver disease (ALD) as the 80% of ingested alcohol is metabolized in the liver [2]. In addition, ethanol is a main ingredient in most of the syrups, tinctures, and other medicines. In small doses it has a great medicinal value. But some people tend to have ethanol abuse [3]. In excess doses, it causes severe hepatic damage in humans and experimental animals. Chronic administration of ethanol is known to have a profound effect on the metabolism of lipids and lipoproteins. Moreover, ethanol is metabolized into cytotoxic acetaldehyde by alcohol dehydrogenase enzyme and acetaldehyde is oxidized to acetate by aldehyde oxidase or xanthine oxidase in the liver, giving rise to reactive oxygen species (ROS) via cytochrome P450 2E1 (CYP 2E1)[4,5]. This leads to oxidative stress in the hepatic cells which is the most striking initial manifestation of alcohol-induced liver injury [6,7]. When there is damage to the liver cell membrane, the cytosolic enzymes are leaked into the blood stream [8]. Therefore, the elevation of these cytosolic enzymes in the blood stream serves as a quantitative marker of hepatic damage.

In recent days, the use of herbal natural products has enhanced world-wide attentions. Many herbal supplements are claimed to assist in healthy lifestyle. Medicinally, herbal drugs have made a significant contribution to the treatment of hepatotoxicity [9]. Alstonia scholaris Linn R.Br. belongs to family Apocynaceae, found in India in the sub Himalayan region from the Yamuna eastward ascending to 3000 feet above sea level, abundantly found in West Bengal and South India.Alstonia scholaris Linn.is known to be a rich source of alkaloids. Amongst the chemical classes present in medicinal plant species, alkaloids stand as a class of major importance in the development of newer drugs because alkaloids possess a great variety of chemical structures and have been identified as responsible for pharmacological properties of medicinal plants. The bark of this plant contains alkaloid ditamine and echitamine, chitenine, echicaoutchin, an amorphous yellow mass, echicerin in acicular crystals, echitin in crystallized scales, echitein in rhombic prisms (a crystallisable acid) and echiretin an amorphous substance, resembling an alkaloid, a fatty acid and fatty resinous substances. An uncrystallisable bitter principle called ditain was isolated and ascribed the febrifuge properties of the drug [10]. The bark of Alstonia scholaris is useful in malarial fevers,abdominal disorders, dyspepsia and in skin diseases [11].The bark is bitter, astringent, digestive, laxative, anthelmintic, antipyretic, stomachic, cardiotonic andtonic [10]. The bark extract has been reported to possessantiplasmodial, immunostimulant, anticancer effect and isalso hepatoprotective [12]. In Ayurveda it is reported that the bark of the plant when soaked

in water overnight, can reduce the blood glucose level after oral administration [13].Bark is also used as febrifuge, depurative andgalactogogue [14]. It is effective in leprosy, skin diseases, pruritis, chronic and foul ulcers, asthma, bronchitis, agalactia and debility12. In folklore medicine, milky juiceis applied on wounds, ulcers and rheumatic pains; mixedwith oil and dropped into ear, it relieves ear ache [14]. Keeping these folkloric claims and reports in view, the present study attempted to evaluate the possible hepatoprotective effects of aqueous extract of Alstonia scholaris bark in paracetamol and ethanol-induced hepatotoxicity in rats.

2. MATERIALS AND METHODS

2.1. Chemicals and drugs

Silymarin was purchased from Sigma-Aldrich labs, Mumbai, India and paracetamol was supplied by Sri Krishna Pharmaceuticals, Hyderabad, A.P, India. Kits for the estimation of selected biochemical parameters such as SGPT, SGOT, ALP, total Bilirubin and total protein were purchased from Coral Clinical systems (Crest biosystems), Goa, India. Oral feeding needles were purchased from BIK industries ltd., Mumbai. All other chemicals and reagents were of analytical grade.

2.2. Extract preparation

Aqueous extract of Alstonia scholaris bark was supplied by Laila neutraceuticals, Vijayawada, A.P, India as gift samples. The extracts were suspended/dissolved in 2% gum acacia suspension prior to administrations.

2.3. Animals study

Adult male albino Wistar rats (150 - 250 g) were obtained from the Mahaveer Enterprizes, Hyderabad, India. They were kept under temperature of (23 ± 2) °C, humidity of 50% and light and dark cycles of 12 h: 12 h. They were fed with commercial pellet diet (Krish scientists Shoppe, Bangalore, India) and water was provided ad libitum. The protocol was approved by Institutional Animal Ethics Committee and the lab was approved by CPCSEA, Government of India (Regd. No. 516/01/A/CPCSEA).

2.5. *In vivo* hepatoprotective study

2.5.1. Paracetamol induced liver damage

Propphylactic study

The rats were divided into six groups, with 6 in each group. The feeding scheme was as follows: Group 1 - Normal control, 2 % w/v gum acacia suspension orally, 1ml/kg once daily for three days Group 2 - Paracetamol as toxicant 2 g/kg orally once daily for 3 days Group 3 - Alstonia scholaris 100 mg/kg orally, 30 min prior to PCM 2 g/kg orally for 3 Days Group 4 - Alstonia scholaris 200 mg/kg orally, 30 min prior to PCM 2 g/kg orally for 3 days Group 5 - Alstonia scholaris 400 mg/kg orally, 30 min prior to PCM 2 g/kg orally for 3 days Group 6 - Silymarin 100 mg/kg orally, 30 min prior to PCM 2 g/kg orally for 3 days

Curative study

Group 1 -Normal control, 2 % w/v gum acacia suspension orally, 1ml/kg once daily for three days

Group 2 -Paracetamol as toxicant 2 g/kg orally once daily for 3 days

Group 3 -Alstonia scholaris 100 mg/kg orally, 30 min prior to PCM 2 g/kg orally for 3 days

Group 4 -Alstonia scholaris 200 mg/kg orally, 30 min prior to PCM 2 g/kg orally for 3 days

Group 5 -Alstonia scholaris 400 mg/kg orally, 30 min prior to PCM 2 g/kg orally for 3 days

Group6 -Silymarin 100 mg/kg orally, 30 min prior to PCM 2 g/kg orally for 3 days.

Oral administration was applied in the study. Silymarin was used as reference hepatoprotective agent. In the preventive study, blood samples were collected on the 0 and 4th days and in the curative study, blood samples were collected on the 0, 26th and 51st days from retro-orbital plexus of rats. Blood samples were centrifuged at 3000 rpm for 30 min. The serum obtained was analyzed for aspartate aminotransferase (AST) [15][19], alanine aminotransferase (ALT) [15] [19], alkaline phosphatase (ALP)[16] [20] and total bilirubin [17][21] using semi-auto analyzer (Screen master-3000) and commercial diagnostic kits. Then on the 4th day in caseof prophylactic study and on the 11th day of curative study, animals were sacrificed and the livers were isolated and washed with fresh saline. Livers were stored in 10% formalin for Prophylactic study.

2.5.2. Ethanol induced hepatotoxicity

Prophylactic study

The rats were divided into six groups, with 6 in each group. The treatment protocol is summarized as given below.

Group 1-Normal control, 2 % w/v gum acacia suspension Orally, 1ml/kg once daily for 28 days

Group 2-Ethanol as toxicant 3.7 g/kg orally once daily for 28 days

Group 3 -Alstonia scholaris 100 mg/kg orally, 30 min prior to ETH 3.7 g/kg orally for 28 days

Group 4 -Alstonia scholaris 200 mg/kg orally, 30 min prior to ETH 3.7 g/kg orally for 28 days

Group 5-Alstonia scholaris 400 mg/kg orally, 30 min prior to ETH 3.7 g/kg orally for 28 days

Group6 -Silymarin 100 mg/kg orally, 30 min prior to ETH 3.7 g/kg orally for 28 days

Curative study

Group 1- Normal control, 2 % w/v gum acacia suspension orally,1ml/kg once daily for 56 days

Group 2- Ethanol as toxicant 3.7 g/kg orally once daily for 28 days followed by 2% gum acacia suspension 1ml/Kg from 29th day to 56th day.

Group 3 - ETH 3.7 g/kg orally for 28 days followed by Alstonia scholaris 100 mg/kg orally from 29th day to 56th day.

Group 4 - ETH 3.7 g/kg orally for 28 days followed by Alstonia scholaris 200 mg/kg orally from 29th day to 56th day.

Group 5 - ETH 3.7 g/kg orally for 28 days followed by Alstonia scholaris 400 mg/kg orally from 29th day to 56th day.

Group6 - ETH 3.7 g/kg orally for 28 days followed by Silymarin100 mg/kg orally from 29thday to 56th day.

In the preventive study, blood samples were collected on the 0 and 29th days and inthe curative study, blood samples were collected on the 0 and 57th days from retro-orbitalplexus of rats. Blood samples were centrifuged at 3000 rpm for 30 min. The serum obtained was analyzed for aspartate aminotransferase (AST)[15][19], alanine aminotransferase (ALT[15]) [19], alkaline phosphatase (ALP)[16] [20] and total bilirubin[17] [21] using semiauto analyzer (Screen master-3000) and commercial diagnostic kits. Then on the 4th day in caseof prophylactic study and on the 11th day of curative study, animals were sacrificed and the livers were isolated and washed with fresh saline. Livers were stored in 10% formalin for histopathological study.

2.7. Data and statistical analysis

All analyses were performed using Graph pad prism version 5.0. All the data was expressed as mean ± SEM. Statistical analysis was performed with one way analysis of variance (1 way ANOVA) followed by Turkey test. P value less than 0.05 was considered to be statistically significant.*=<0.05, **=P<0.01 and ***=P<0.001, when compared with toxicant group.

3. RESULTS

3.1. Determination of serum biochemical parameters

(a) Paracetamol induced hepatotoxicity

Results presented in Table 1, Table 2, Table 4 and Table 5 indicated that levels of serum enzymes, namely AST, ALT, ALP and total bilirubin, were significantly (P < 0.01) increased in paracetamol treated rats compared with normal rats. However, levels of serum enzymes, like AST, ALT, ALP and total bilirubin, were significantly (P < 0.01) decreased in rats treated with AEAS and Silymarin compared to Paracetamol treated rats in both prophylactic and curative studies.

Table No:1- Basal levels of serum biochemical parameters in rats for PCM induced hepatotoxicity on 0 day (Prior to treatment) in Prophylactic study

G	TREATMENT	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TOTAL PROTEIN (g/dl)	TOTAL BILIRUBIN (mg/dl)
1	2% Gum acacia(1ml/kg;p.o)	74.79±2.35	26.49±2.10	149.70±10.69	7.25±0.70	0.62±0.09
2	PCM (2g/kg; p.o)	82.20±3.68	28.57±2.04	144±5.81	7.72±0.33	0.601±0.074
3	PCM + Alstonia scholaris(2g/kg; p.o + 100mg/kg;p.o)	78.02±3.82	27.74±1.42	152.30±9.45	7.25±0.39	0.58±0.07
4	PCM + Alstonia scholaris (2g/kg; p.o + 200mg/kg;p.o)	84.38±4.06	32.24±1.30	156.65±5.35	7.50±0.45	0.59±0.06
5	PCM + Alstonia scholaris (2g/kg; p.o + 400mg/kg;p.o)	76.37±3.51	32.54±3.44	175.71±5.33	7.65±0.67	0.84±0.09
6	PCM + Silymarin (2g/kg; p.o + 100mg/kg;p.o)	86.62±2.11	33.74±1.41	183.21±3.55	7.34±0.48	0.705±0.21

Values are expressed as Mean ±SEM

Table No: 2- Influence of different plant extracts on serum biochemical parameters in rats for PCM induced hepatotoxicity on 4th day of treatment (Prophylactic study)

G	TREATMENT	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TOTAL PROTEIN(g/dl)	TOTAL BILIRUBIN (mg/dl)
1	2%**Gumacacia**(1 ml/kg;p.o)	74.95±2.76	26.06±1.97	158.67±10.66	7.33±0.86	0.79±0.10
2	PCM (2g/kg; p.o)	436.79±25.15	136.01±5.04	725.60±25.95	4.76±0.33	1.75±0.38
3	PCM + **Alstonia scholaris** (2g/kg;p.o+100mg/kg;p.o)	190.66±12.30***	111.98±6.09*	362.50±29.06***	6.04±0.47	1.57±0.31
4	PCM + **Alstoniascholaris** (2g/kg;p.o+200mg/kg;p.o)	133±4.89***	97.83±4.75***	318.30±29.60***	5.93±0.56	0.57±0.05***
5	PCM + **Alstoniascholaris** (2g/kg;p.o+400mg/kg;p.o)	95.79±2.19***	40.66±2.85***	242.26±24.99***	6.85±0.65	0.45±0.18***
6	PCM + **Silymarin** (2g/kg;p.o+100mg/kg;p.o)	87.59±2.01***	35.82±1.74***	198.06±6.51***	7.10±0.49*	0.77±0.22**

Values are expressed as Mean ±SEM

Table No: 3- Average % change in serum biochemical parameters in rats for PCM induced hepatotoxicity on 4th day of treatment (Prophylactic study)

G	TREATMENT	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TOTAL PROTEIN (g/dl)	TOTAL BILIRUBIN (mg/dl)
1	2% ***Gum acacia***(1ml/kg;p.o)	0.214	1.623	5.99	1.10	27.42
2	PCM (2g/kg; p.o)	431.30↑	376.06↑	403.89↑	38.34↓	191.18↑
3	PCM + ***Alstonia scholaris*** (2g/kg; p.o + 100mg/kg;p.o)	144.40↓	303.68↓	138.02↓	16.69↑	170.69↓
4	PCM + ***Alstonia scholaris*** (2g/kg; p.o + 200mg/kg;p.o)	57.6↓	203.44↓	103.19↓	20.93↑	3.39↓
5	PCM + ***Alstonia scholaris*** (2g/kg; p.o + 400mg/kg;p.o)	25.43↓	24.95↓	37.87↓	10.46↑	46.43↓

6	PCM + ***Silymarin*** (2g/kg; p.o + 100mg/kg;p.o)	1.12↓	6.16↓	8.10↓	3.27↑	9.22↓

Table No: 4- Basal levels of serum biochemical parameters in rats for PCM induced hepatotoxicity on 0 day (Prior to treatment) in Curative study

G	TREATMENT	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TOTAL PROTEIN (g/dl)	TOTAL BILIRUBIN (mg/dl)
1	2% Gum acacia (1ml/kg; p.o)	88.59±3.35	34.49±1.85	189.02±5.57	6.99±0.35	0.67±0.06
2	PCM (2g/kg; p.o)	81.25±2.54	33.28±1.86	189.30±4.23	7.36±0.72	0.74±0.07
3	PCM + Alstonia scholaris (2g/kg; p.o + 100mg/kg; p.o)	88.8±4.30	32.18±1.78	182.56±2.16	7.71±0.63	0.72±0.06
4	PCM + Alstonia scholaris (2g/kg; p.o + 200mg/kg; p.o)	80.53±1.80	28.78±1.25	174.85±2.20	7.00±0.73	0.57±0.12
5	PCM + Alstonia scholaris (2g/kg; p.o + 400mg/kg; p.o)	90.56±2.70	36.10±2.08	193.56±3.11	7.98±0.65	0.66±0.08
6	PCM + Silymarin (2g/kg; p.o + 100mg/kg; p.o)	89.21±2.25	35.69±1.57	190.26±1.15	9.20±0.52	0.88±0.09

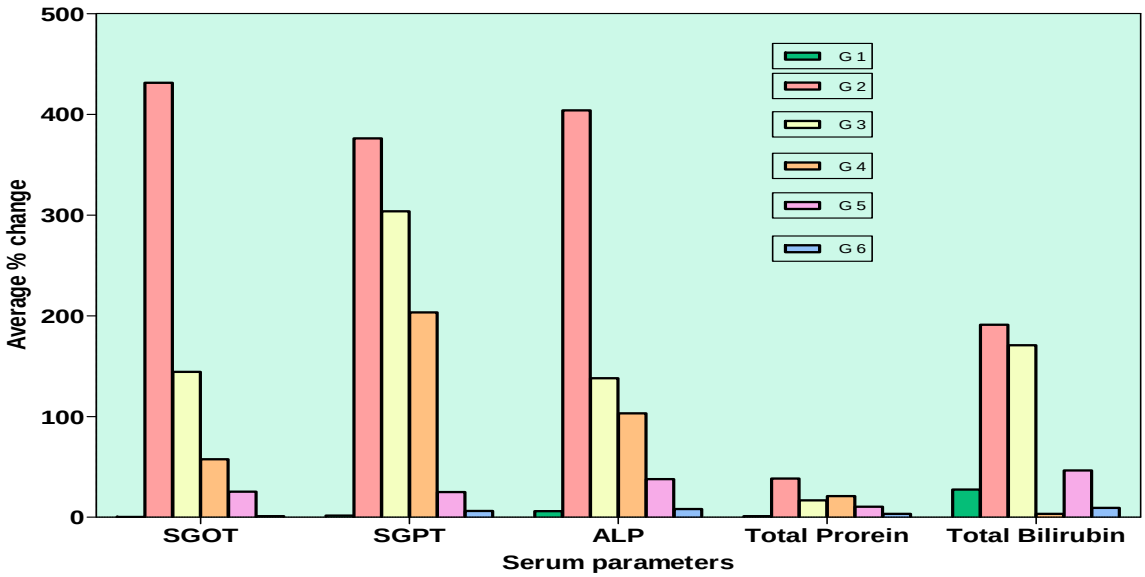
Table No: 5- Influence of different plant extracts on serum biochemical parameters in rats for PCM induced hepatotoxicity on 11th day of treatment (Curative study)

G	TREATMENT	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TOTAL PROTEIN (g/dl)	TOTAL BILIRUBIN (mg/dl)
1	2% Gum acacia(1ml/kg;p.o)	89.51±3.51	34.51±2.07	188.32±3.35	7.09±0.28	1.12±0.23
2	PCM (2g/kg; p.o)	405.57±4.08	127.94±6.41	564.26±34.81	4.27±0.52	1.10±0.12
3	PCM + Alstonia scholaris (2g/kg; p.o + 100mg/kg;p.o)	189.31±11.80***	109.41±7.50	321.99±21.90***	6.89±0.53*	2.61±1.44
4	PCM + Alstonia scholaris (2g/kg; p.o + 200mg/kg;p.o)	122.09±3.16***	72.57±3.60***	274.54±21.09***	6.00±0.46	0.53±0.06
5	PCM + Alstonia scholaris (2g/kg; p.o + 400mg/kg;p.o)	105.37±5.24***	45.22±3.34***	204.56±5.05***	7.49±0.73**	0.70±0.05

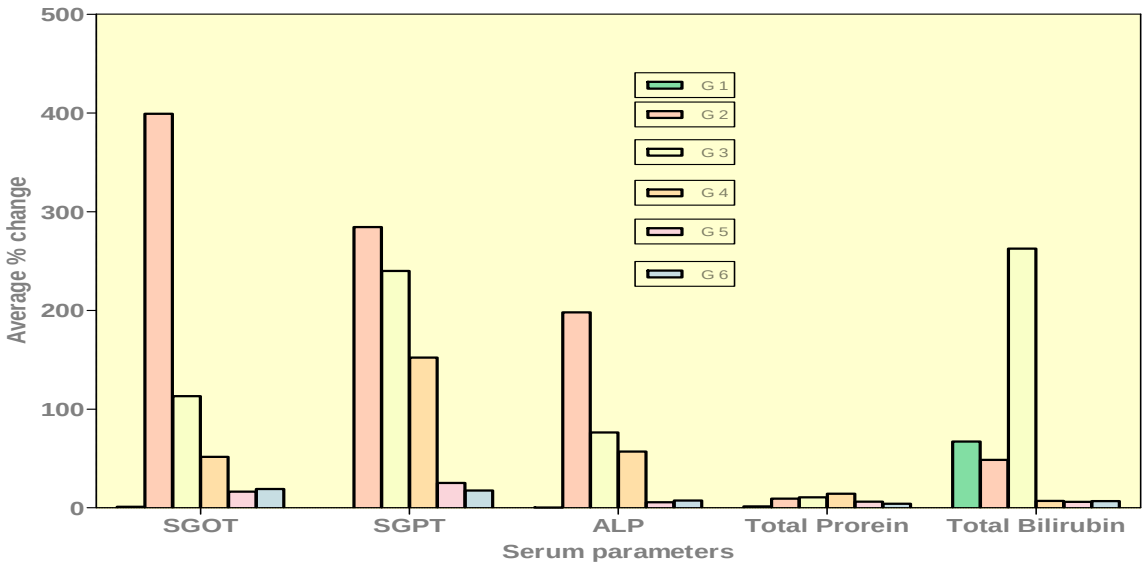
6	PCM + Silymarin (2g/kg; p.o + 100mg/kg;p.o)	106.28±5.59***	41.98±1.77***	204.12±7.20***	8.82±0.56***	0.82±0.08

Table No: 6- Average % change in serum biochemical parameters in rats for PCM induced hepatotoxicity on 11th day of treatment (Curative study)

G	TREATMENT	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TOTAL PROTEIN (g/dl)	TOTAL BILIRUBIN (mg/dl)
1	2% Gum acacia(1ml/kg;p.o)	1.04	0.058	0.37	1.41	67.16
2	PCM (2g/kg; p.o)	399.16↑	284.43↑	198.08↑	9.36↓	48.65↑
3	PCM + *Alstonia scholaris* (2g/kg; p.o + 100mg/kg;p.o)	113.18↓	239.99↓	76.37↓	10.64↑	262.50↓
4	PCM + *Alstonia scholaris* (2g/kg; p.o + 200mg/kg;p.o)	51.60↓	152.15↓	57.01↓	14.28↑	7.02↓
5	PCM + *Alstonia scholaris* (2g/kg; p.o + 400mg/kg;p.o)	16.35↓	25.26↓	5.68↓	6.14↑	6.06↓
6	PCM + Silymarin (2g/kg; p.o + 100mg/kg;p.o)	19.13↓	17.62↓	7.28↓	4.13↑	6.82↓



Histogram No: 1-Average % change of Serum biochemical parameters in PCM induced hepatotoxicity in rats (Prophylactic study)



Histogram No: 2- Average % change of Serum biochemical parameters in PCM induced hepatotoxicity in rats (Curative study)

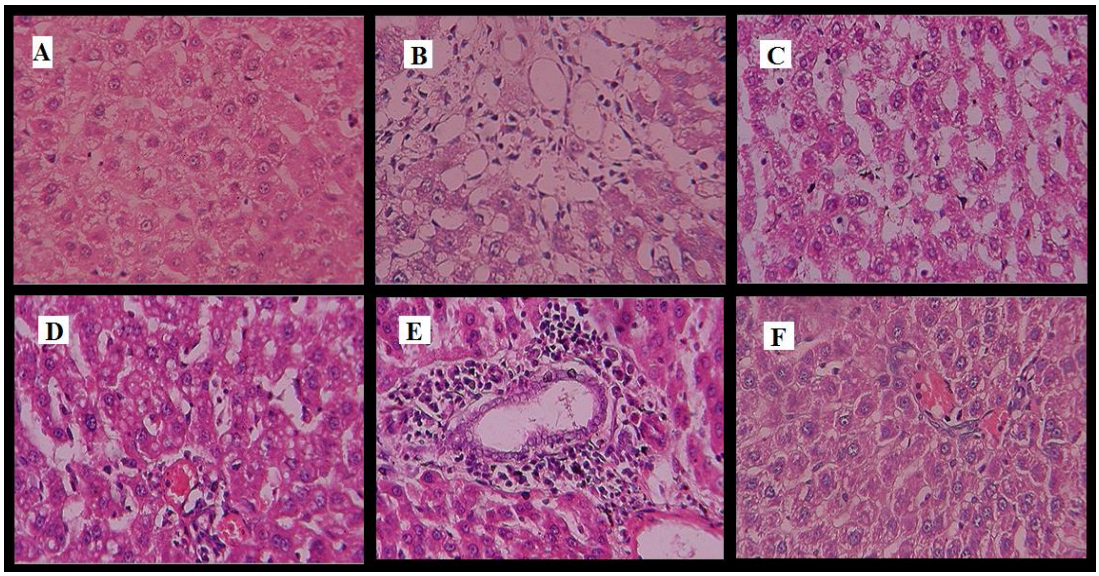


Fig. No.1- Liver architectures in Prophylactic study (PCM induced liver damage): (A) normal control [2% Gum acacia]; (B) toxic control [PCM (2g/kg)]; (C) PCM + AEAS (2g/kg + 100mg/kg); (D) PCM + AEAS (2g/kg + 200mg/kg); (E) PCM + AEAS (2g/kg + 400mg/kg); (F) PCM + Silymarin (2g/kg + 100mg/kg)

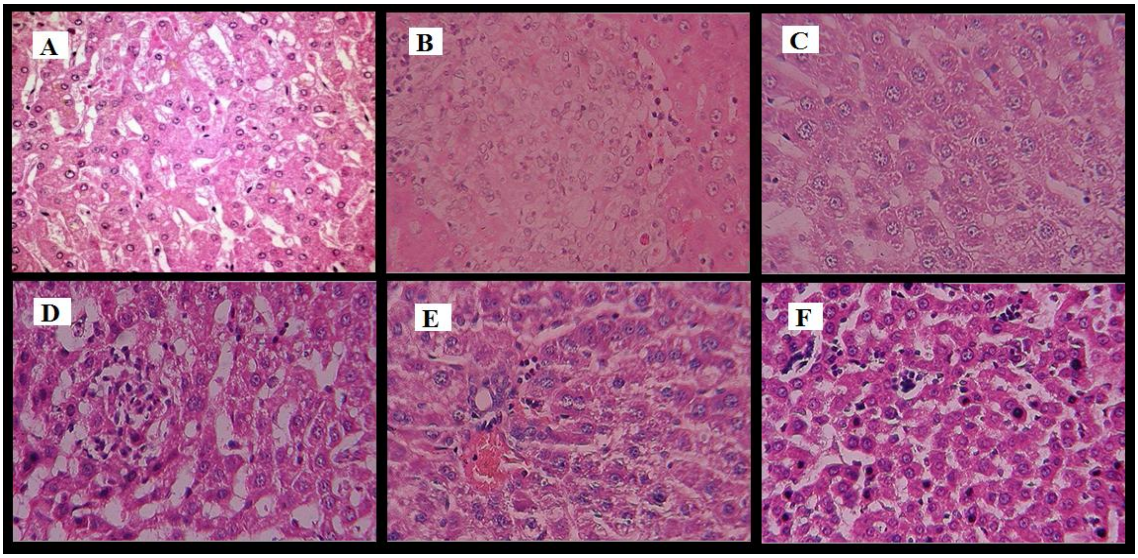


Fig. No.2- Liver architectures in Curative study (PCM induced liver damage): (A) normal control [2% Gum acacia]; (B) toxic control [PCM (2g/kg)]; (C) PCM + AEAS (2g/kg + 100mg/kg); (D) PCM + AEAS (2g/kg + 200mg/kg); (E) PCM + AEAS (2g/kg + 400mg/kg); (F) PCM + Silymarin (2g/kg + 100mg/kg)

Ethanol induced hepatotoxicity

Results presented in Table 7, Table 8, Table 10 and Table 11 indicated that levels of serum enzymes, namely AST, ALT, ALP and total bilirubin, were significantly ($P < 0.01$) increased in ethanol treated rats compared with normal rats. However, levels of serum enzymes, like AST, ALT, ALP and total bilirubin, were significantly ($P < 0.01$) decreased in rats treated with AEAS and Silymarin compared to ethanol treated rats in both prophylactic and curative studies.

Table No: 7- Basal levels of serum biochemical parameters in rats for ETH induced hepatotoxicity on 0 day (Prior to treatment) in Prophylactic study

G	TREATMENT	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TOTAL PROTEIN (g/dl)	TOTAL BILIRUBIN (mg/dl)
1	2% Gum acacia(1ml/kg;p.o)	88.91±2.79	32.79±1.85	185.40±2.54	8.68±0.52	0.83±0.05
2	ETH (3.7g/kg;p.o)	99.42±3.43	34.52±2.22	189.20±3.00	73.85±0.87	0.79±0.05
3	ETH + Alstonia scholaris (3.7g/kg;p.o + 100mg/kg;p.o)	91.96±3.02	33.11±2.27	190.13±3.08	8.89±0.33	0.85±0.05
4	ETH + Alstonia scholaris (3.7g/kg; p.o + 200mg/kg;p.o)	84.10±5.23	30.50±3.26	181.45±3.61	7.87±0.65	0.86±0.06
5	ETH + Alstonia scholaris (3.7g/kg; p.o +	85.42±6.43	32.53±2.93	180.09±4.11	8.40±0.65	0.88±0.66

	400mg/kg;p.o)					
6	ETH + Silymarin (3.7g/kg; p.o + 100mg/kg;p.o)	9.64±1.96	33.34±1.83	187.31±2.64	8.41±0.06	0.83±0.04

Table No: 8- Influence of different plant extracts on serum biochemical parameters in rats for ETH induced hepatotoxicity on 29th day of treatment (Prophylactic study)

G	TREATMENT	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TOTAL PROTEIN (g/dl)	TOTAL BILIRUBIN (mg/dl)
1	2% Gum acacia (1ml/kg; p.o)	89.33±3.06	33.43±2.01	186.68±2.68	8.74±0.62	0.85±0.07
2	ETH (3.7g/kg; p.o)	402.99±5.03	119.62±7.91	509.32±20.09	4.78±0.48	1.02±0.05
3	ETH + Alstonia scholaris (3.7g/kg; p.o + 100mg/kg; p.o)	230.27±31.62***	114.38±5.01	403.21±5.53***	7.17±0.43	0.74±0.10
4	ETH + Alstonia scholaris (3.7g/kg; p.o + 200mg/kg; p.o)	142.81±13.47***	94.39±3.54*	311.58±20.96***	6.86±0.73	0.75±0.08
5	ETH + Alstonia scholaris (3.7g/kg; p.o + 400mg/kg; p.o)	109.03±3.72***	49.38±5.49***	200.18±4.13***	8.12±0.61**	0.64±0.05**
6	ETH + Silymarin (3.7g/kg; p.o + 100mg/kg; p.o)	95.16±2.17***	36.66±3.06***	180.48±15.75***	8.03±0.62**	0.41±0.08***

Table No: 9- Average % change in serum biochemical parameters in rats for ETH **induced hepatotoxicity on 29th day of treatment (Prophylactic study)**

G	TREATMENT	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TOTAL PROTEIN (g/dl)	TOTAL BILIRUBIN (mg/dl)
1	2% Gum acacia(1ml/kg;p.o)	47.24	1.95	0.69	0.69	2.40
2	ETH (3.7g/kg; p.o)	150.40↑	246.52↑	169.19↑	40.17↓	29.11↑
3	ETH + *Alstonia scholaris* (3.7g/kg; p.o + 100mg/kg;p.o)	75.33↓	245.45↓	112.07↓	19.35↑	12.94↓
4	ETH + *Alstonia scholaris* (3.7g/kg; p.o + 200mg/kg;p.o)	69.81↓	209.47↓	71.72↓	12.83↑	13.95↓

5	ETH + *Alstonia scholaris* (3.7g/kg; p.o + 400mg/kg;p.o)	27.64↓	51.79↓	11.16↓	3.33↑	27.27↓
6	ETH + Silymarin (3.7g/kg; p.o + 100mg/kg;p.o)	18.01↓	9.96↓	3.65↓	4.52↑	50.60↓

Table No: 10- Basal levels of serum biochemical parameters in rats for ETH induced hepatotoxicity on 0 day (Prior to treatment) in Curative study

G	TREATMENT	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TOTAL PROTEIN(g/dl)	TOTAL BILIRUBIN(mg/dl)
1	2% Gum acacia (1ml/kg;p.o)	84.4±4.32	31.51±1.50	177.62±7.86	8.02±0.86	0.79±0.09
2	ETH (3.7g/kg; p.o)	91.12±6.50	31.78±3.85	183.52±3.73	9.57±0.96	0.85±0.07
3	ETH + Alstonia scholaris (3.7g/kg; p.o + 100mg/kg;p.o)	88.43±2.41	32.72±1.86	180.66±5.77	6.94±0.73	0.57±0.09
4	ETH + Alstonia scholaris (3.7g/kg; p.o + 200mg/kg;p.o)	84.41±2.42	33.00±2.35	180.50±4.51	8.45±0.65	0.74±0.08
5	ETH + Alstonia scholaris (3.7g/kg; p.o + 400mg/kg; p.o)	85.50±4.77	31.59±2.42	182.63±4.58	8.44±0.71	0.90±0.07
6	ETH + Silymarin (3.7g/kg; p.o + 100mg/kg; p.o)	85.81±2.99	29.91±2.40	186.09±10.27	9.03±1.01	0.80±0.06

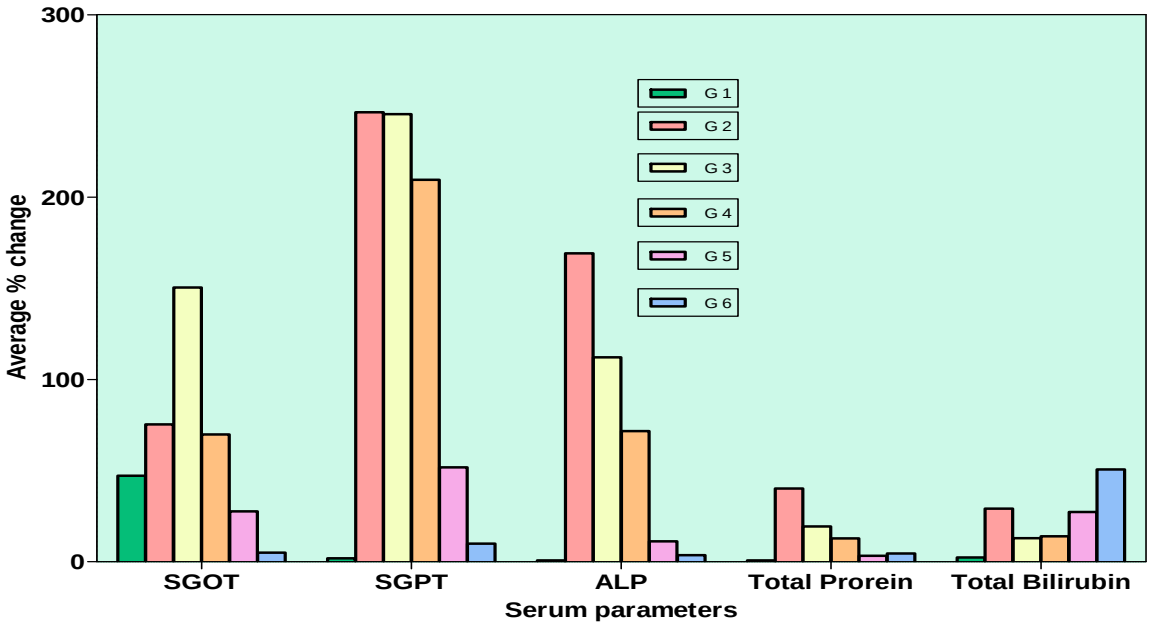
Table No: 11- Influence of different plant extracts on serum biochemical parameters in rats for ETH induced hepatotoxicity on 57th day of treatment

G	TREATMENT	SGOT(IU/L)	SGPT (IU/L)	ALP (IU/L)	TOTAL PROTEIN(g/dl)	TOTAL BILIRUBIN (mg/dl)
1	2% Gum acacia (1ml/kg; p.o)	87.45±4.95	33.72±1.93	181.91±6.12	8.41±0.80	0.74±0.11
2	ETH (3.7g/kg; p.o)	380.82±19.10	96.30±4.55	401.59±8.79	5.40±0.57	0.92±0.10
3	ETH + Alstonia scholaris (3.7g/kg; p.o + 100mg/kg; p.o)	208.37±28.41***	120.37±7.44**	307.61±19.93**	5.62±0.69	0.61±0.08*
4	ETH + Alstonia scholaris	131.46±3.78***	78.77±4.09	250.27±14.95***	7.59±0.56	0.81±0.07

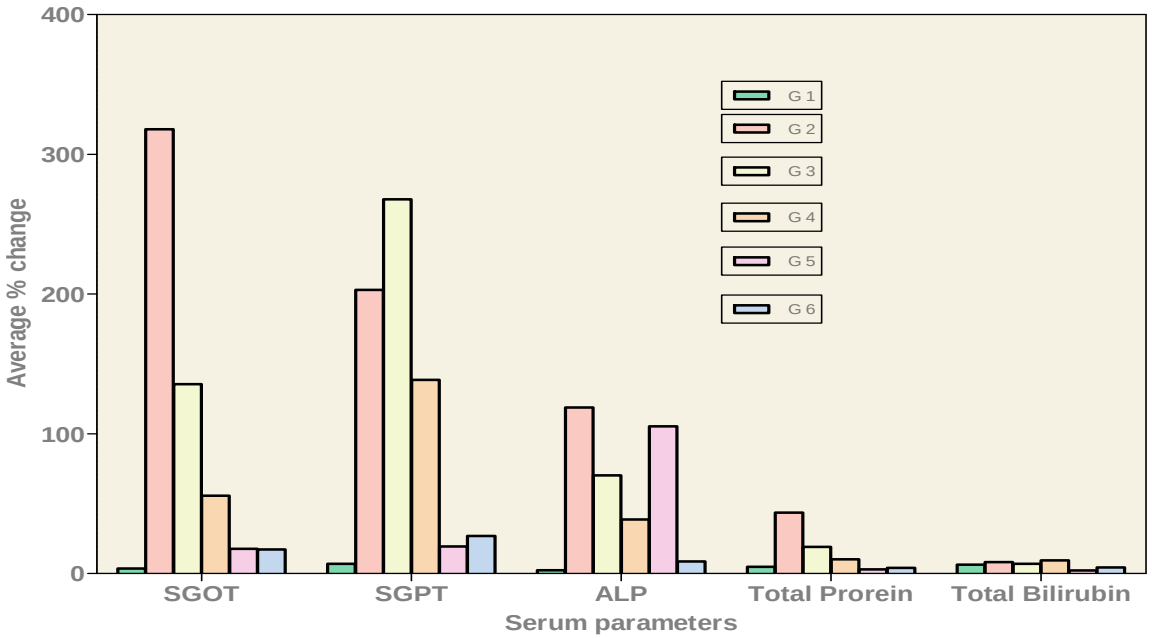
	(3.7g/kg; p.o + 200mg/kg; p.o)					
5	ETH + Alstonia scholaris (3.7g/kg; p.o + 400mg/kg; p.o)	100.66±4.66***	37.72±2.29***	201.87±6.00***	8.19±0.59	0.88±0.05
6	ETH + Silymarin (3.7g/kg; p.o + 100mg/kg; p.o)	100.55±3.63***	37.95±2.59***	202.16±10.49***	8.66±0.79*	0.45±0.08***

Table No: 12- Average % change in serum biochemical parameters in rats for ETH **induced hepatotoxicity on 57th day of treatment.**

G	TREATMENT	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TOTAL PROTEIN (g/dl)	TOTAL BILIRUBIN (mg/dl)
1	2% Gum acacia(1ml/kg;p.o)	3.61	7.01	2.41	4.86	6.33
2	ETH (3.7g/kg; p.o)	317.93	203.02	118.83	43.57	8.24
3	ETH + Alstonia scholaris (3.7g/kg; p.o + 100mg/kg;p.o)	135.63	267.88	70.26	19.02	7.02
4	ETH + Alstonia scholaris (3.7g/kg; p.o + 200mg/kg;p.o)	55.74	138.69	38.65	10.18	9.46
5	ETH + Alstonia scholaris (3.7g/kg; p.o + 400mg/kg;p.o)	17.73	19.40	105.35	2.96	2.22
6	ETH + Silymarin (3.7g/kg; p.o + 100mg/kg;p.o)	17.18	26.88	8.64	4.09	4.37



Histogram No: 3- Average % change of Serum biochemical parameters in ETH induced hepatotoxicity in rats (Prophylactic study)



Histogram No: 4- Average % change of Serum biochemical parameters in ETH induced hepatotoxicity in rats (Curative study)

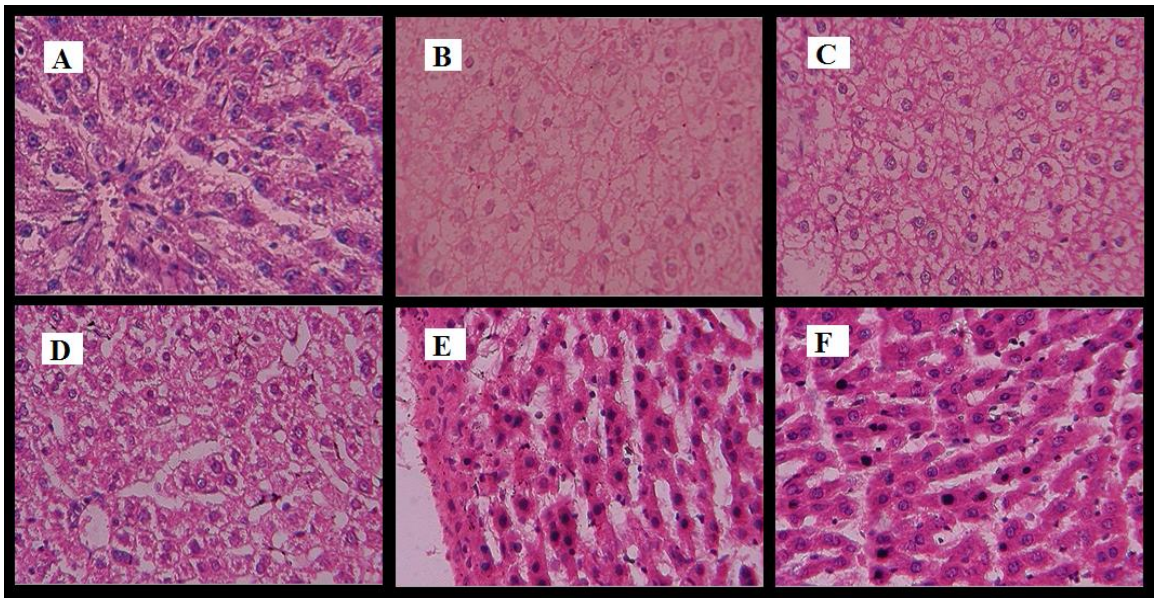


Fig. No.3- Liver architectures in Prophylactic study (ETH induced liver damage): (A) normal control [2% Gum acacia]; (B) toxic control [ETH (3.7g/kg; p.o)]; (C) ETH + AEAS (3.7g/kg + 100mg/kg); (D) ETH 3.73.7 + AEAS (3.7g/kg + 200mg/kg); (E) ETH + AEAS (3.7g/kg + 400mg/kg); (F) ETH + Silymarin (3.7g/kg + 100mg/kg)

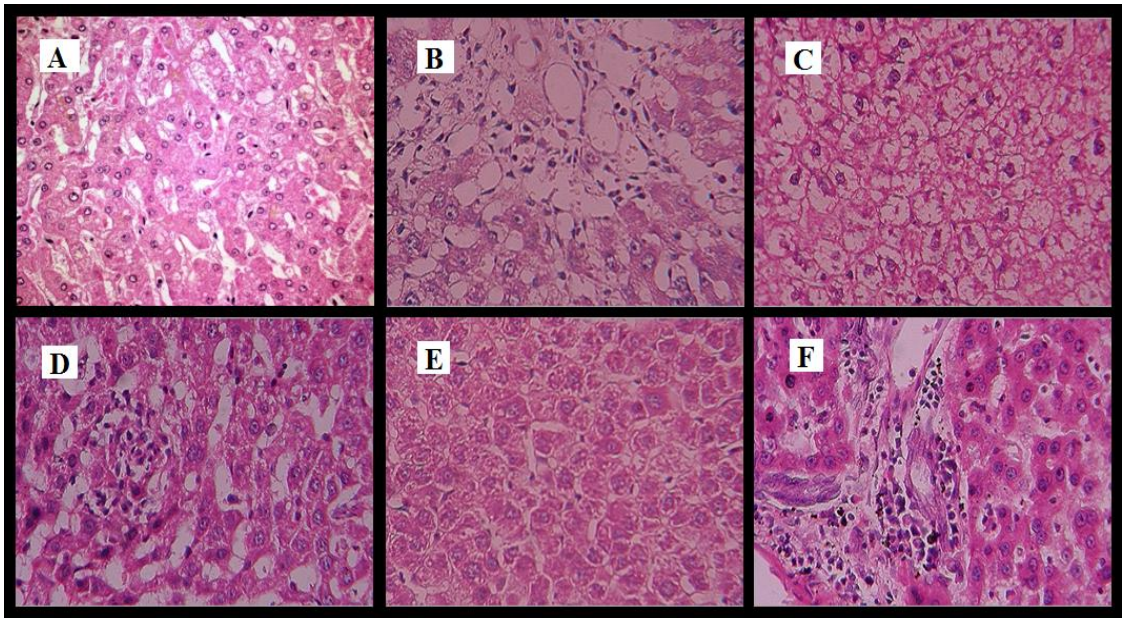


Fig. No.4- Liver architectures in Curative study (ETH induced liver damage): (A) normal control [2% Gum acacia]; (B) toxic control [ETH (3.7g/kg; p.o)]; (C) ETH + AEAS (3.7g/kg; p.o + 100mg/kg); (D) ETH + AEAS (3.7g/kg; p.o + 200mg/kg); (E) ETH + AEAS (3.7g/kg; p.o + 400mg/kg); (F) ETH + Silymarin (3.7g/kg; p.o + 100mg/kg)

4. DISCUSSION

Liver participates in a variety of enzymatic metabolic activities. Administration of ethanol causes elevation of serum ASP, ALT, ALP and total bilirubin levels in rats, indicating that ethanol may induce hepatocellular damages which in turn alters the structure and function of liver cells [18] [22] and [19][23]. Our study on the ethanol induced hepatic damage are in accordance with previous reports [20][24]. Silymarin is a standardized extract of the milk thistle (Silybum marianum) chiefly contains flavonoid, includings silybin, silybinin, silydianin and silychristin [21][25]. Silymarin offers good protection in various toxic models of experimental liver diseases in laboratory animals. It functions through mechanisms of antioxidative, anti-lipid peroxidative, antifibrotic, anti-inflammatory, membrane stabilizing, immunomodulatory and liver regenerating [22][26]. Silymarin has been applied in alcoholic liver diseases, liver cirrhosis, Amanita mushroom poisoning, viral hepatitis, toxic and drug induced liver diseases and in diabetic patients in clinical settings. Silymarin may also be a useful hepatoprotection drug for hepatobiliary diseases and hepatotoxicity induced by drugs. Moreover, it is used as a standard drug and exhibited potent hepatoprotective activity within the dose range of 25 to 200 mg/kg [23][27] and [24] [28].It was reported that lipid peroxidation and oxidative stress were implicated in PCM induced hepatic damage. Due to

malignant infiltration and cirrhosis of liver, serum ALT and AST levels are significantly increase due to leakage of these enzymes from cytosol of liver [25].

The increase in ALP levels are due to damage of cell membrane of tissue where the enzymes are firmly attached. Hepatic necrosis or membrane damage release these enzymes into circulations and can be measured in serum. ALT (SGPT) is a more specific enzyme for assessing liver damage and also a better parameter than AST (SGOT) which is a non specific enzyme to the liver [26]. The high level of SGOT indicates liver damage, Cardiac infraction and muscles injury [27]. The rise in ALT is an indication of PCM induced specific hepatotoxicity where the rise in AST levels indicates hepatic, non-hepatic damage. Elevated levels of serum enzymes are indicative of loss of cellular leakage and loss of functional integrity of hepatic cellular membrane [25]. Additionally the rise in ALP and Bilirubin levels is an indication of the functional change in hepatocytes as a result of hepatic damage. A significance decrease in protein levels in serum was observed in PCM induced hepatotoxicity in rats due to the transfer of protein to other parts of the body. The PCM treatment alters the incorporation of amino acids to synthesize proteins [28] and also due to the decrease in hepatic DNA, RNA and total protein levels. Silymarin has significantly reduced the rise in serum biochemical parameters like ALT,AST,ALP,Total Biilirubin and increased total protein in PCM induced heapatotoxic rats both in prophylactic and curative studies. Kurma S Rao and Mishra 1997 reported the similar effects with Silymarin in PCM induced hepatotoxic rats [29].

Ethanol induced drug metabolizing microsomal enzyme CYP2E1 accelerate alcohol metabolism with a result increase in acetaldehyde production [30]. The 4-10 fold CYP2E1 induction due to alcohol results in enhanced lipid peroxidation and increased rate of reactive oxygen species (ROS) formation [30, 31] which results in the formation of more free radicals causing enhanced hepatic cell damage. The peroxidative decomposition of membrane lipid initiated by oxygen free radicals is one of the important mechanism in alcohol induced liver damage [31].

Alcohol induced tissue damage is mainly based on the toxicity of its major metabolite, acetaldyhyde. The increased formation of ROS such as Hydrogen Peroxide and super oxide anaions have been implicated as causative factors involved in various forms of chronic liver diseases [32].

This study demonstrated that Alstonia scholaris aqueous bark extract had reduced levels of AST, ALT and ALP which were elevated by paracetamol and ethanol administration. The results were in accordance with the findings of other investigators [33] [13]. Our results are consistent with earlier studies, which strongly suggest that Alstonia scholaris may protect the structural integrity of hepatocytes and prevent the release of cytosolic enzymes into bloodstream.

The Histopathological studies supported the biochemical evidence for the hepatoprotective activity of the selected plant bark aqueous extracts. The normal hepatic cell is a polygonal cell and Binucleated with nucleolus and abundant cytoplasm(Naidu RS et al., 2007). The above features were found in normal control groups. In PCM treated and ETH treated groups, in both prophylactic and curative studies histopathology showed very high fatty and vaculolar degeneration, necrosis, derangement of crods and cellular infiltration. Alstonia scholaris showed good reduction in these abnormalities with regeneration

The Phytochemicals present in the plant bark extract i.e Alstonia scholaris include alkaloids, ditamin, and echitamine, echitein (a crystallisable acid), echiretin, an alkaloid, a fatty acid, linalool (a terpeniod). The presence of alkaloids, Flavonoids, polyphenols, glycosides, terpenoids, steroids, saponins and vitamins might be responsible for the antioxidant and hepatoprotective activity [10]

5. CONCLUSIONS

The present study demonstrated that the aqueous extract of Alstonia scholaris bark is protective against ethanol-induced as well as paracetamol induced hepatotoxicity which might be due to its antioxidant potential against DPPH, hydroxyl and superoxide radicals. The hepatoprotective role of Alstonia scholaris extract at a dose of 400 mg/kg body weight was found to be comparable with Silymarin which might be due to the presence of flavonoids and alkaloids.

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