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Hepatoprotective activity of alcoholic and aqueous extracts of bark of *Bassia Latifolia Roxb*. against paracetamol induce hepatotoxicity in rats

Rizwan A. Sheikh[1]*, D. Jeevan Mani Babu[2], N. Venkat Rao[2], Petricia Regena Irene[2], Sachin More[1], Ashish Turaskar[1]

[1]*Manoharbhai Patel Institute of Pharmacy, Gondia, Maharashtra, India*
[2]*V.L. College of Pharmacy, Raichur-584103, Karnataka, India*

ABSTRACT

In litreture plant Bassia latifolia is widely mentioned for its uses in ulcers, fractures, bronchitis, ear complaints, expelling worms from the body and in orchitis. To evaluate the hepatoprotective activity of alcoholic and aqueous extracts of bark of Bassia latifolia (AEBBL and AQEBBL) (Sapotaceae) in PCM induced hepatotoxicity in rats.Hepatoprotective activity of the AEBBL and AQEBBL was evaluated against PCM (curative aspect) induced hepatotoxicity model in rats, Standard drug like Silymarin was used as reference in each model.Preliminary phytochemical studies with AEBBL and AQEBBL revealed the presence of phytoconstituents like glycosides, tanins, saponins, triterpenoids and flavonoids in both the extracts. When this extracts is subjected for LD_{50} studies, produced abnormal behaviour or mortality even at the dose level of 2000 mg/Kg body weight in mice. Three different doses like low 1/20th (100 mg/Kg), medium 1/10th (200 mg/Kg) and high 1/5th (400 mg/Kg) doses from the maximum dose tested for LD_{50} were selected for the present study.Silymarin, AEBBL and AQEBBL treated groups when compared to PCM (curative aspect) induced hepatotoxic rats the increased TST, wet liver weight and wet liver volume, ALT, AST, ALP, BILD, BILT, CHO and TG levels were significantly reduced and ALB and PRO levels were significantly increased. The histopathological changes i.e. fatty changes (steatosis), necrosis etc were partly or fully prevented. The present study on hepatoprotective activity with AEBBL and AQEBBL confirmed the above mentioned effect because of several phytoconstituents like triterpenoids and flavonoids as these were already reported for their hepatoprotective activity.

Keywords: *Bassia latifolia* (Roxb), Bark, Aqueous extracts, Alcoholic extract PCM, Hepatoprotective, and Silymarin.

INTRODUCTION

Liver is the heaviest and the second largest gland and also a key organ regulating homeostasis in the body. Liver cells called hepatocytes, every second perform several complex biochemical and a number of important functions, including bile production, excretion of bilirubin, cholesterol, hormones and drugs. It is also responsible for metabolism of fats, proteins, carbohydrates, enzyme activation, storage of glycogen, vitamins, minerals and synthesis of plasma proteins such as albumin, globulin and clotting factors.

Toxic liver injury produced by drugs and chemicals are similar to natural liver disease. Continuous use of agents like paracetamol, tetracycline, antitubercular drugs, oral contraceptives of hormonal origin, chemicals used as food preservatives and agrochemicals are threatening the integrity of liver. Further addiction of alcohol and other drugs aggravated the problem and malnutrition also an important cause of liver damage[1].

Drug induced liver injury is an unresolved problem and often limits drug therapy in clinical practice. Liver injury follows with the inhalation, ingestion or parenteral administration of a number of chemical and pharmacological agents.[2] The nature and extent of liver damage varies depending on the type of stage of its disease. Not all liver diseases produce the same patterns of change, but many forms of chronic liver disease will ultimately lead to the typical clinical and histological picture of cirrhosis. The effects of liver disease on hepatic metabolism of drugs are complex and difficult to predict, particularly when multiple drugs are administered simultaneously.[3]

It is well known that drugs are structurally altered in the liver to form biologically inactive or active or toxic metabolites. Indiscriminate use of analgesics[4], antimalarials[5], anti-tubercular drugs[6], oral contraceptives, antidepressants, anticonvulsants[7] etc. are potential threats to the integrity of liver. Quite often certain drugs even in therapeutic dose may cause hepatic damage in susceptible individuals. Toxic effects of drugs on the liver or its function may mimic any naturally occurring hepatic disease. The spectrum of drug induced liver injury ranges from asymptomatic increase in enzyme (markers of hepatic damage) levels to fulminant hepatic failure. It can occur in different forms including acute drug-induced hepatitis, steatohepatitis, cholestasis, chronic hepatitis and may lead to liver failure. Many drugs may cause more than one type of hepatic injury.[8]

About 20,000 deaths found every year due to liver disorders and hepatocellular carcinoma is one of the 10 most common tumors in the world with over 2,50,000 new cases each year. In India, about 40 polyherbal commercial formulations are being used for hepatoprotection. It has been reported that 160 phytoconstituents from 101 plants have hepatoprotective activity.[1] Liver protective herbals contain a variety chemical constituents like phenols, coumarins, lignans, essential oils, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthenes. Plant extracts of many crude drugs are also used for the treatment of liver disorders. Extracts of 25 different plants have been reported to cure liver disorders.[9]

In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulates liver function, offer protection to the liver from damage or help regeneration of hepatic cells except silybon, a recent synthetic drug.[10] however there are numbers of drugs employed in traditional system of medicine for liver affections.[11]

1.1 Objective:

The present study was undertaken to investigate the effect of B. latifolia against PCM induced hepatoxicity in rats.[12, 13]

MATERIALS AND METHODS

2.1. Experimental animals [14]

Albino rats (Wistar strain) of either sex weighing between 150-200 g and Albino mice of either sex (16-20 g) were procured from National Centre for Laboratory Animal Sciences, C/0 Sri Venkateswara Enterprises,Bengaluru for experimental purpose. All the animals were acclimatized for 7 days under standard husbandry condition. i.e;

Room temperature	-	26 ± 2^0 C
Relative humidity	-	45-55%
Light/ dark cycle	-	12:12 h

The animals were fed with a synthetic standard pellet diet from Amrut Laboratories & Pranav Agro Industries Ltd, Sangli (MH) and water allowed *ad libitum* under strict hygienic conditions. All animal studies were performed in accordance to the Guidelines of CPCSEA (Registration Number 557/02/c/CPCSEA 18th Feb, 2002) and Institutional Animal Ethical Committee (IAEC) of V.L. College of Pharmacy, Raichur (Karnataka) and all the procedures were followed as per rules and regulations.

2.2 Drugs

Bark powder of Bassia latifolia, Paracetamol, Silymarin were used as standard reference in the present study.

2.3 Parameters studied during the study of hapatoprotective activity in ALC induced hepatotoxic rats.

A. Functional parameters

Thiopentone induced sleeping time (TST)

B. Physical parameters

a. Wet liver weight
b. Wet liver volume

C. Biochemical parameters

a. Serum alanine aminotransferase (ALT) or Serum glutamate pyruvate transaminase (SGPT)

b. Serum aspartate aminotransferase (AST) or Serum glutamate oxaloacetate transaminase (SGOT)
c. Serum alkaline phosphatase (ALP)
d. Serum direct bilirubin (BILD)
e. Serum total bilirubin (BILT)
f. Serum total proteins (TP)
g. Serum albumin (ALB)
h. Serum cholesterol (CHO)
i. Serum triglycerides (TG)

D. Histopathological studies of the liver sections.

2.4. Preparation of aqueous extract[15]

A. Preparation of alcoholic extract

The bark powder packed in Soxhlet apparatus was extracted with 95 % alcohol for 18 h and appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extract was then transferred into a previously weighed empty beaker, then it was kept on a water bath maintained at 50^{o}C and evaporated to a thick paste. The extract AEBBL was thoroughly air dried to remove all traces of the solvent, then the percentage yield was calculated.

B. Preparation of aqueous extract

About 100 g bark powder of *B.latifolia* was taken into a round bottom flask (2000 ml) and macerated with into 500 ml of distilled water and 10 ml of chloroform (preservative) for 24 h with occasional shaking for every hour. Then the marc was removed by filtering the extract, and then the extract (AQEBBL) was concentrated on a water bath maintained at 50^{o}C.

The AEBBL and AQEBBL extracts were examined for their colour and consistency and their percentage yield was calculated with reference to the quantity used for extraction. These two extracts were stored in airtight containers in a refrigerator below 10^{o}C.

2.5 Drug Protocol

Paracetamol induced hepatotoxicity[16, 17] (Curative aspect)

Albino rats weighing between 150-200 g each group containing 6 animals were divided into 9 groups.

Group A - *Normal control (vehicle treated p.o) for 10 days*
Group B - *Toxicant (paracetamol 2 g/Kg, p.o for 3 days and from 4th-10th day vehicle only*
Group C - *Paracetamol 2 g/Kg daily p.o for 3 days and Standard Silymarin 100 mg/Kg, p.o from 4th-10th day*
Group D - *Paracetamol 2 g/Kg daily p.o for 3 days, AEBBL Low dose (100 mg/Kg) p.o from 4th-10th day.*
Group F - *Paracetamol 2 g/Kg daily p.o for 3 days, AEBBL Medium dose (200 mg/Kg) p.o from 4th-10th day*
Group G - *Paracetamol 2 g/Kg daily p.o for 3 days, AQEBBL Low dose (100 mg/Kg) p.o from 4th-10th day.*
Group H - *Paracetamol 2 g/Kg daily p.o for 3 days, AQEBBL Medium dose (200 mg/Kg) p.o from 4th-10th day.*
Group I - *Paracetamol 2 g/Kg daily p.o for 3 days, AQEBBL Higher dose (400 mg/Kg) p.o from 4th-10th day.*

Experimental procedure:

Wistar rats weighing between (150-200 g) were divided into 9 groups of 6 rats in each. Group A was served as normal control which was given with vehicle only. Group B with Paracetamol (2 gm/Kgp.o). Group C with Silymarin (100 mg/Kg p.o) that serves as standard. Animals in groups D, E and F were treated with three different doses (low, medium and high) of AEBBL and groups G, H and I with AQEBBL Groups B, C, D, E, F, G, H and I were intoxicated with Paracetamol (2 gm/Kg p.o) for 3 days and from 4th-10th day with different doses of AEBBL & AQEBBL in related groups respectively. On the 11th day, after recording thiopentone sodium sleeping time in all groups of animals. They were anaesthetized with ether. Blood was collected through retroorbital puncture, later sacrificed by overdose of ether. Livers removed were washed with saline, weighed and stored in 10 % Formaldehyde for histological studies.

2.7 STATISTICAL ANALYSIS

All the recorded results will be expressed as mean ± SEM from 6 animals. Statistical difference in mean will be analyzed by using one-way ANOVA (analysis of variance) followed by Dunnett's't' test. P< 0.05*,0.01** and 0.001*** will be considered as statistically significant.

RESULTS

Effect of AEBBL and AQEBBL on PCM induced hepatoxicity in rats (Curative aspect)

A. Thiopentone induced sleeping time, physical and biochemical parameters in normal control rats

In normal control rats various parameters are recorded as i.e. TST (68.33 ±0.84 min), physical parameters like wet liver weight (5.05 ±0.21 g/100 g), wet liver volume (6.27 ±0.12 ml/100 g) and biochemical parameters like ALT (55.57 ±1.89 U/L), AST (119.91 ±3.12 U/L), ALP (122.12 ±3.30 U/L), BILD (0.31 ±0.03 mg/dL), BILT (0.37 ±0.03 mg/dL), ALB (5.59 ±0.43 g/dL), PRO (15.28 ±1.08 g/dL), CHO (144.71 ±2.94 mg/dL) and TG (43.02 ±0.98 mg/dL). The results are tabulated in Table No. 1 & 2 and Fig Nos. 1-12.

In normal group liver sections showed the normal lobular architecture of the liver. (Fig. No. 13)

B. Effect of PCM on TST, physical and biochemical parameters in rats

When compared to normal control animals TST (129.33 ±1.67 min), physical parameter like wet liver weight (6.91 ±0.03 g/100 g) and wet liver volume (7.58 ±0.12 ml/100 g) and other biochemical parameters like ALT (167.25 ±8.22 U/L), AST (242.54 ±20.51 U/L), ALP (253.51 ±4.41 U/L), BILD (0.80 ±0.08 mg/dL), BILT (1.74 ±0.16 mg/dL), CHO (242.79 ±2.64 mg/dL) and TG (209.92 ±5.76 mg/dL) are significantly increased whereas ALB (3.49 ±0.24 g/dL) and PRO (9.37 ±0.26 g/dL) are significantly decreased. The results are tabulated in Table No. 1 & 2 and Fig Nos. 1 - 12. Histological studies revealed that liver show severe vacuolar degeneration, moderate inflammatory cell infiltration, congested vessels. (Fig. No. 14)

C. Effect of Silymarin on TST, physical and biochemical parameters in PCM induced hepatotoxic rats

When compared to toxicant control, Silymarin treated animals have shown significant reduction in TST (82.00 ±0.86 min) and physical parameters like wet liver weight (5.50 ±0.09 g/100 g) , wet liver volume (6.72 ±0.20 ml/100 g) and other biochemical parameters like ALT (71.50 ±2.51 U/L), AST (133.16 ±1.43 U/L), ALP (140.31 ±0.86 U/L), BILD (0.47 ±0.01 mg/dL), BILT (0.66 ±0.02 mg/dL), CHO (181.05 ±3.13 mg/dL) and TG (97.79 ±2.61 mg/dL). Whereas ALB (5.27 ±0.24 g/dL) and PRO (12.85 ±0.26 g/dL), are significantly increased. The results are tabulated in Table No. 1 & 2 and Fig Nos. 1-12. Histology of liver showed no microvascular fatty changes and mild centeral venous congestion. (Fig. No. 15)

D. Effect of AEBBL on TST, physical and biochemical parameters in PCM induced hepatotoxic rats

When compared to toxicant control, animals treated with AEBBL (low, medium, high doses) have shown dose dependent hepatoprotective activity as seen with significant reduction in TST min, physical parameters like wet liver weight (g/100 g), wet liver volume (ml/100 g) and biochemical parameters like ALT, AST, ALP (U/L), BILD, BILT, CHO and TG (mg/dL). ALB and PRO (g/dL) levels are significantly increased. The results are tabulated in Table No. 1 & 2 and Fig Nos. 1-12.

Histology of liver revealed,

AEBBL Low dose (100 mg/kg) + PCM treated group: The sections from the liver, showed severe vacuolar degeneration, mild inflammatory cell infiltration, congested vessels.

AEBBL Medium dose (200 mg/kg) + PCM treated group: Sections from the liver, showed severe vacuolar degeneration, very mild perivascular edema,congested vessels.

AEBBL High dose (400 mg/kg) + PCM treated group: The liver sections showed mild vacuolar degeneration,congested vessels. (Fig. No. 16)

E. Effect of AQEBBL on TST, physical and biochemical parameters in PCM induced hepatotoxic rats

When compared to toxicant control animals, AQEBBL treated groups have shown a dose dependent hepatoprotective activity as TST min, physical parameters like wet liver weight (g/100 g), wet liver volume (ml/100 g) and biochemical parameters like ALT, AST, ALP (U/L), BILD, BILT, CHO and TG (mg/dL) levels are significantly reduced with med and high dose and similarly ALB and PRO (g/dL) levels are significantly increased. The results are tabulated in Table No. 1 & 2 and Fig Nos. 1-12.

Histology of liver revealed,

AQEBBL Low dose (100 mg/kg) + PCM treated group: The sections from the liver, showed Severe vacuolar degeneration,very mild inflammatory cell reaction,very mild perivascular edema and congested vessels.

AQEBBL Medium dose (200 mg/kg) + PCM treated group: Sections from the liver, showed Mild perivascular edema,moderate vacuolar degeneration,very mild inflammatory cell reaction, congested vessels.

AQEBBL High dose (400 mg/kg) + PCM treated group: The sections from the liver, showed very mild perivascular edema and very mild inflammatory cell reaction. (Fig. No. 17)

Table No.1 Effect of AEFLC and AQEFLC on biochemical parameters in paracetamol induced hepatotoxic rats

Serum biochemical parameter	Normal	Standard	Toxicant	AEBBL 100 mg/kg	AEBBL 200 mg/kg	AEBBL 400 mg/kg	AQEBBL 100 mg/kg	AQEBBL 200 mg/kg	AQEBBL 400 mg/kg
ALT	54.25±2.06	61.45±3.37***	146.62±2.50***	141.37±2.79 ns	131.81±1.64***	89.92±2.55***	135.85±1.93*	90.5±1.78***	71.08±2.26***
AST	120.35±3.20	140±2.21***	259.87±2.46***	248.3±2.20 ns	196.65±2.17***	161.23±2.81***	240.28±10.59*	181.77±3.26***	151.4±2.50***
ALP	121.6±2.36	126.67±2.13***	262.54±2.75***	242.95±2.80**	214±5.38***	168.52±3.23***	249.72±8.06 ns	151.08±1.94***	132.75±2.83***
BILD	0.23±0.04	0.38±0.04***	2.01±0.22***	1.63±0.24 ns	0.95±0.15***	0.52±0.01***	1.59±0.24 ns	0.8±0.02***	0.49±0.02***
BILT	0.39±0.06	0.6±0.02***	2.38±0.13***	2.06±0.26 ns	1.28±0.14***	0.98±0.06***	1.96±0.14 ns	0.82±0.03***	0.74±0.03***
ALB	5.85±0.07	5.68±0.15***	3.47±0.26***	3.74±0.07 ns	4.17±0.07**	4.76±0.07***	3.89±0.06 ns	4.53±0.10***	5.23±0.13***
PRO	25.15±0.90	21.21±0.72***	13.05±0.38***	12.21±0.59 ns	18.26±2.37*	20.5±1.69***	14.51±1.34 ns	17.78±0.58*	21±0.33***
CHO	141.15±1.68	154.47±1.22***	236.23±5.28***	224.37±4.58 ns	190.82±2.68***	177.27±5.04***	208.69±15.40 ns	187.36±5.65***	160.11±10.09***
TG	92.27±2.60	104.22±3.25***	215.7±2.91***	190.6±18.17 ns	169.3±4.0***	142.92±1.15***	182.75±1.05**	158.27±2.97***	111.52±3.90***

AEBBL-Alcoholic extract of bark of B.latifolia, AQEBBL- Aqueous extract of bark of B.latifolia

Table No. 2 Effect of AEBBL and AQEBBL on thiopentone induced sleeping time (TST), wet liver weight and volume in paracetamol induced hepatotoxic rats

Physical parameter	Normal	Standard	Toxicant	AEBBL 100 mg/kg	AEBBL 200 mg/kg	AEBBL 400 mg/kg	AQEBBL 100 mg/kg	AQEBBL 200 mg/kg	AQEBBL 400 mg/kg
Duration of sleep (min)	**68.33±0.84**	**129.33±1.67*****	**82.00±0.86*****	**126.0 ±1.39 ns**	**106.00±1.06*****	**92.67±1.12*****	**124.33 ±1.98 ns**	**99.33±1.84*****	**89.83±0.75*****
Liver weight (g/100 g)	**5.05±0.21**	**6.91±0.03*****	**5.50±0.09*****	**6.68 ±0.08 ns**	**5.95±0.17*****	**5.68±0.23*****	**6.57 ±0.07 ns**	**5.99±0.06*****	**5.60±0.16*****
Liver volume (ml/100 g)	**6.27±0.12**	**7.58±0.12*****	**6.72±0.20****	**7.53 ±0.15 ns**	**7.08 ±0.25 ns**	**6.77±0.15****	**7.03 ±0.19 ns**	**6.90±0.11***	**6.78±0.17****

n = 6, Significant at P < 0.05, 0.01** and 0.001***, ns = not significant*
AEBBL-Alcoholic extract of bark of B.latifolia, AQEBBL- Aqueous extract of bark of B.latifolia

n = 6, Significant at P < 0.05, 0.01** and 0.001***, ns = not significant,*

Fig. No. 1 Effect of AEBBL and AQEBBL on thiopentone induced sleeping time (TST) in Paracetamol induced hepatotoxic rats (Curative aspect)

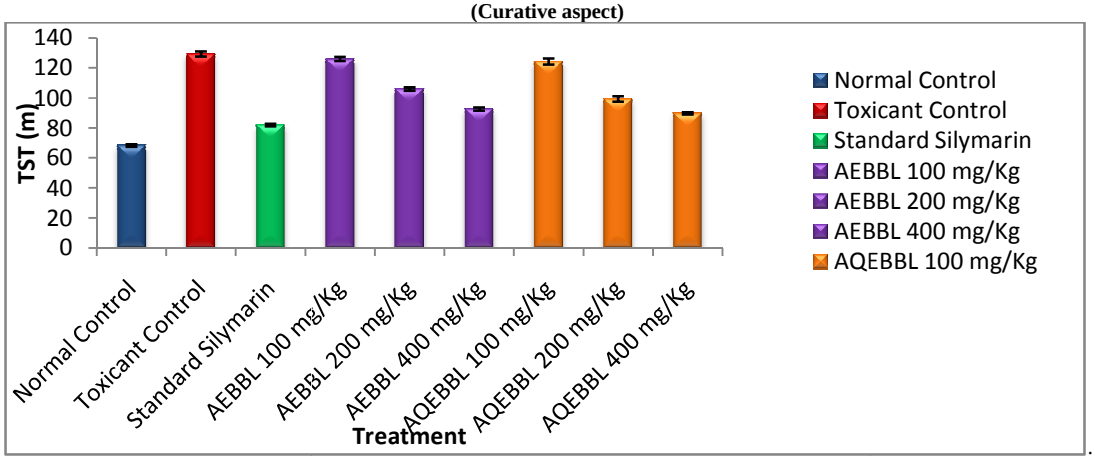


Fig. No. 2 Effect of AEBBL and AQEBBL on wet liver weight in Paracetamol induced hepatotoxic rats (Curative aspect)

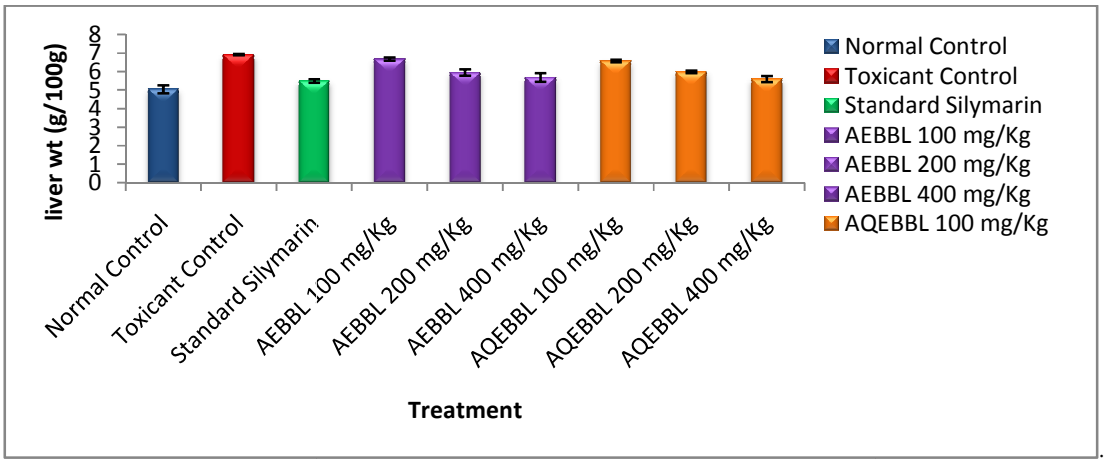


Fig. No. 3 Effect of AEBBL and AQEBBL on wet liver volume in Paracetamol induced hepatotoxic rats (Curative aspect)

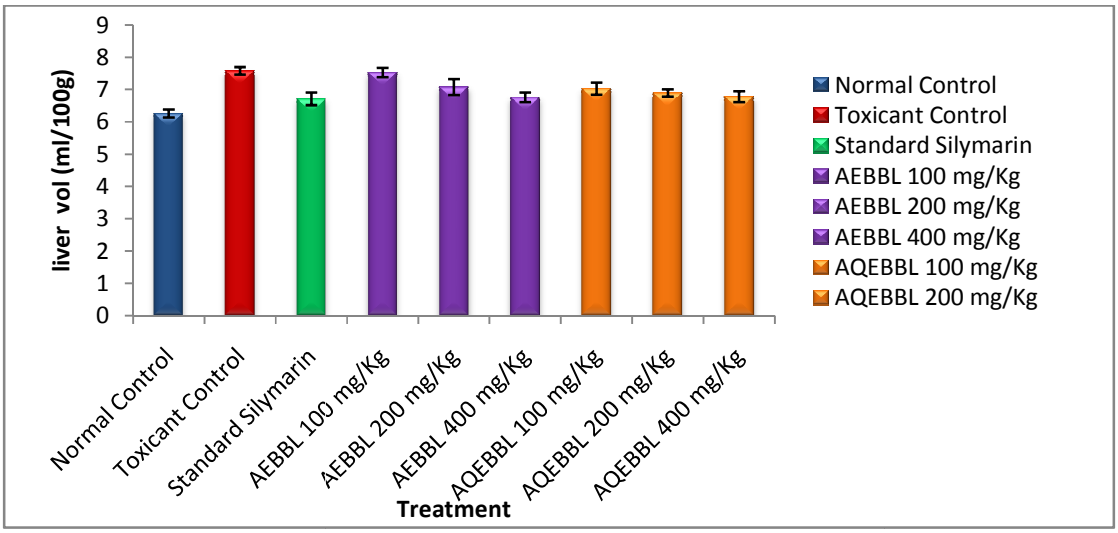


Fig. No. 4 Effect of AEBBL and AQEBBL on serum ALT levels in Paracetamol induced hepatotoxic rats (Curative aspect)

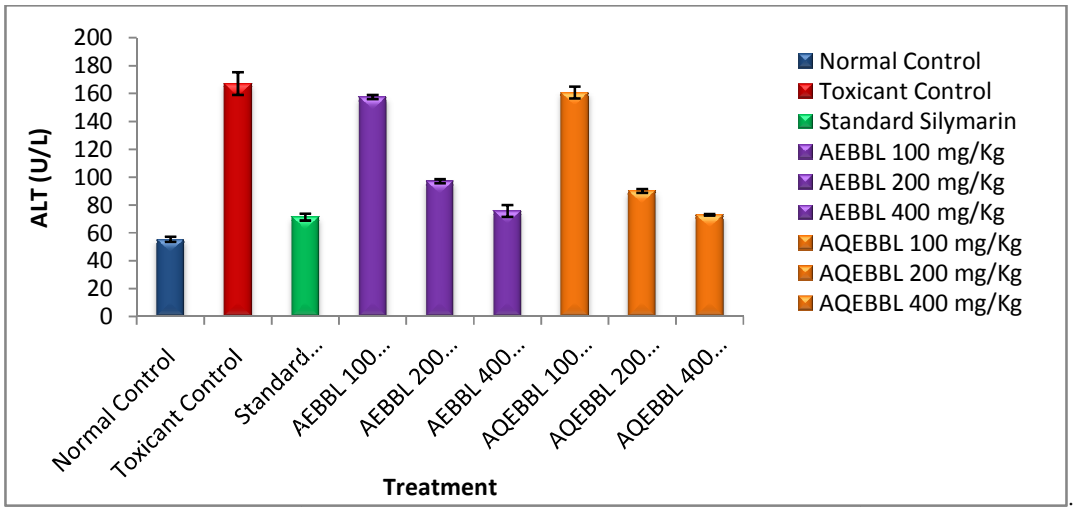


Fig. No. 5 Effect of AEBBL and AQEBBL on serum AST levels in Paracetamol induced hepatotoxic rats (Curative aspect)

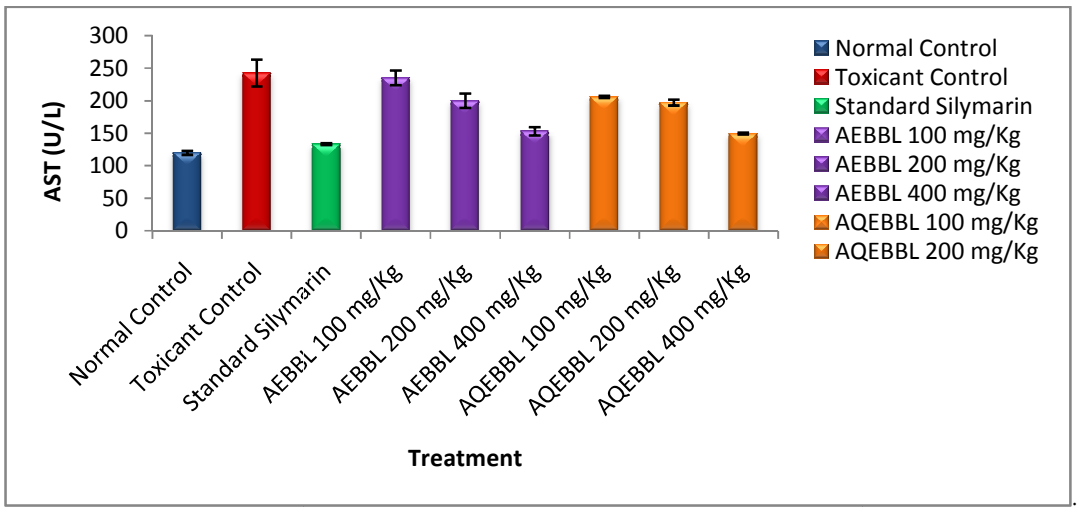


Fig. No. 6 Effect of AEBBL and AQEBBL on serum ALP levels in Paracetamol induced hepatotoxic rats (Curative aspect)

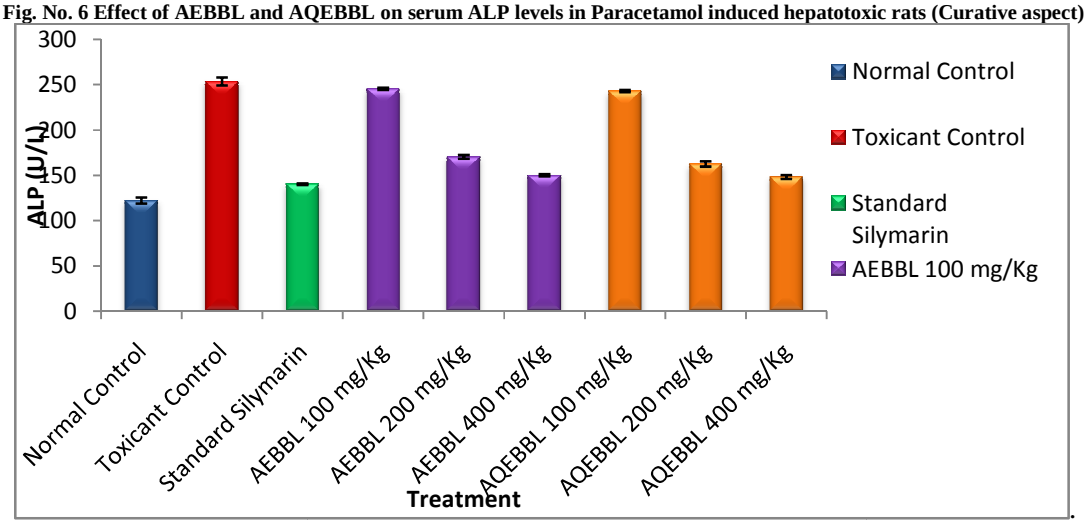


Fig. No. 7 Effect of AEBBL and AQEBBL on serum BILD levels in Paracetamol induced hepatotoxic rats (Curative aspect)

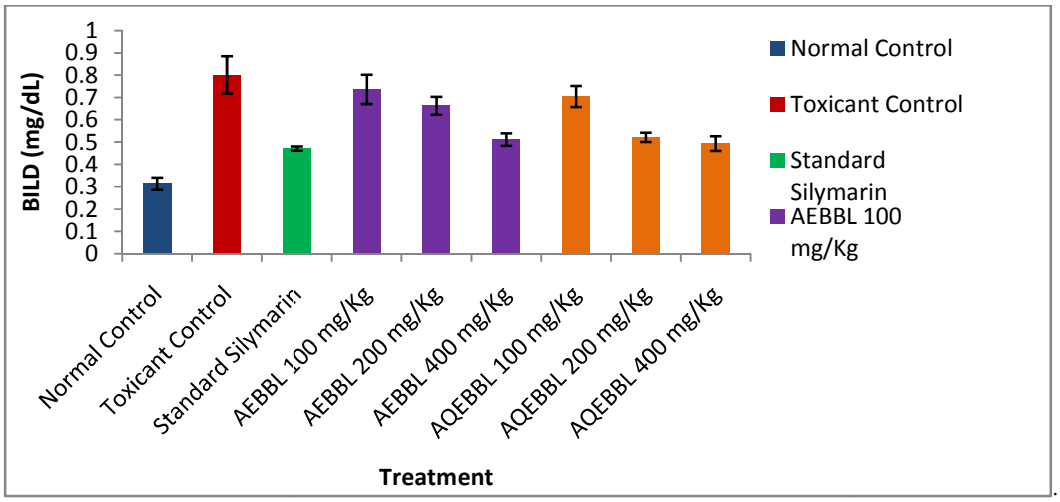


Fig. No. 8 Effect of AEBBL and AQEBBL on serum BILT levels in Paracetamol induced hepatotoxic rats (Curative aspect)

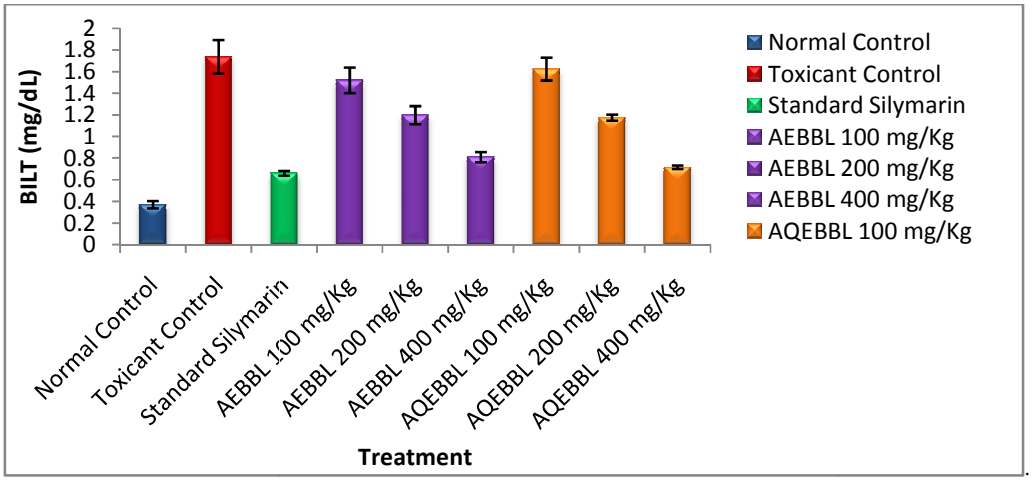


Fig. No. 9 Effect of AEBBL and AQEBBL on serum ALB levels in Paracetamol induced hepatotoxic rats (Curative aspect)

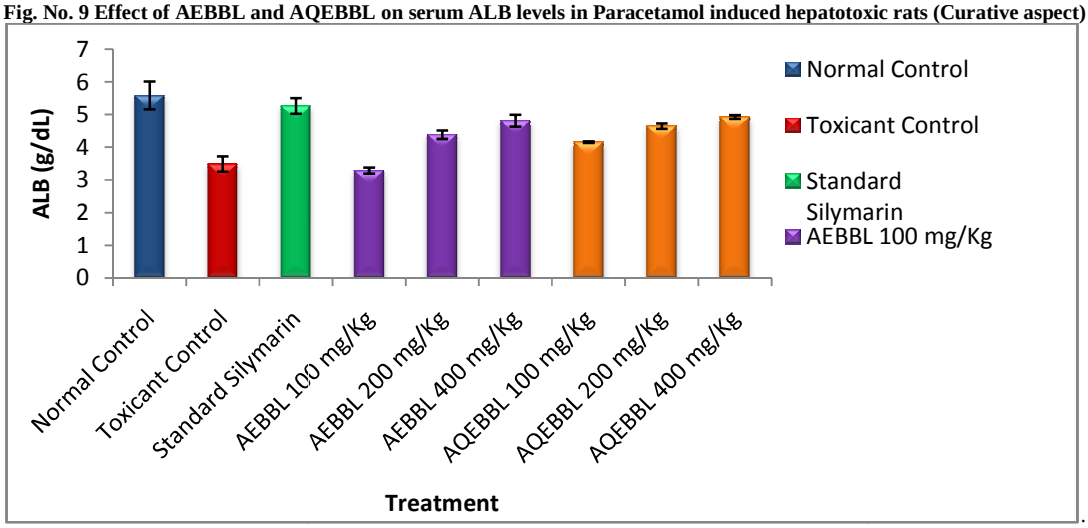


Fig. No. 10 Effect of AEBBL and AQEBBL on serum TP levels in Paracetamol induced hepatotoxic rats (Curative aspect)

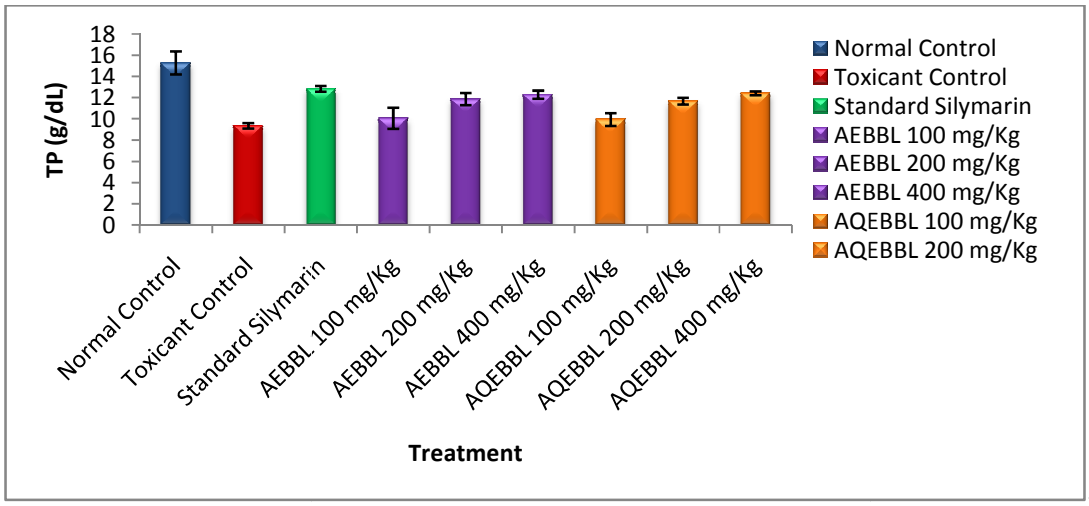


Fig. No. 11 Effect of AEBBL and AQEBBL on serum CHO levels in Paracetamol induced hepatotoxic rats (Curative aspect)

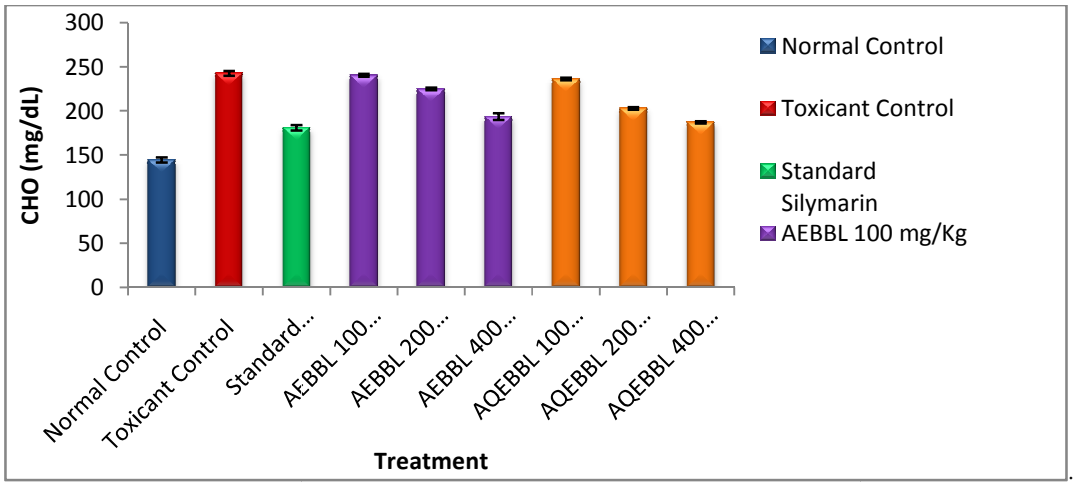


Fig. No. 12 Effect of AEBBL and AQEBBL on serum TG levels in Paracetamol induced hepatotoxic rats (Curative aspect)

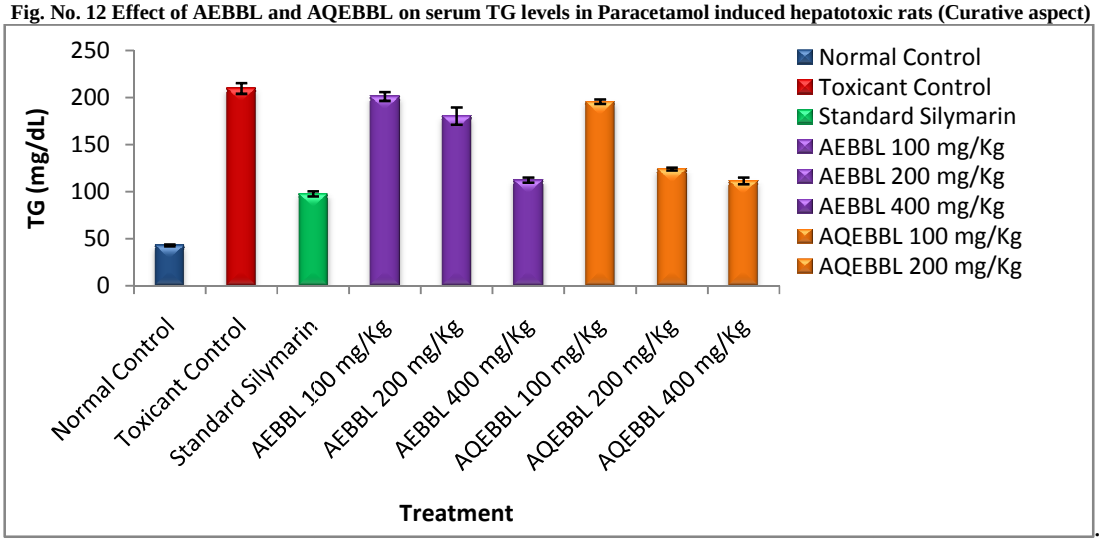


Fig. No. 13. Histology of normal hepatic tissue

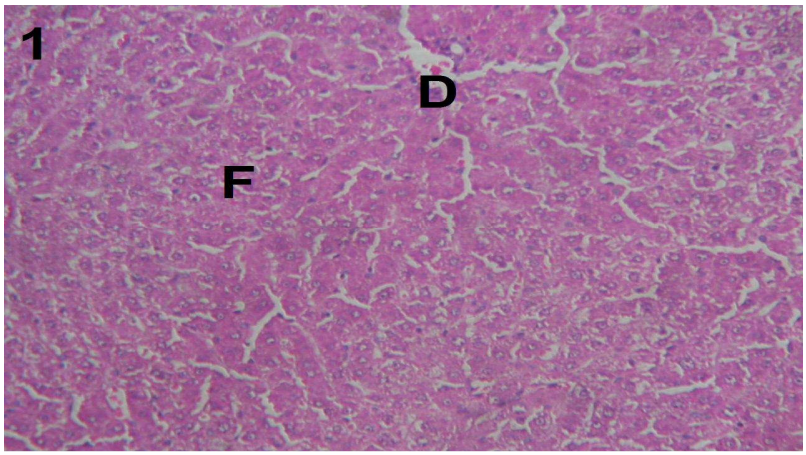


Fig. No. 14. PCM induced damage in hepatic tissue

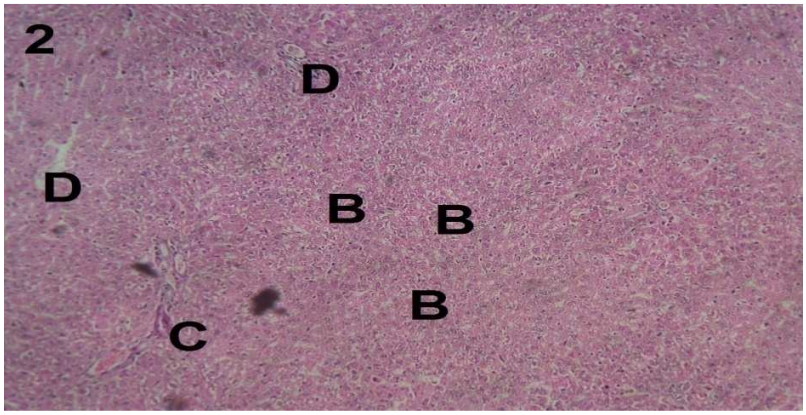


Fig. No. 15. Effect of Silymarin on PCM induced hepatic damage

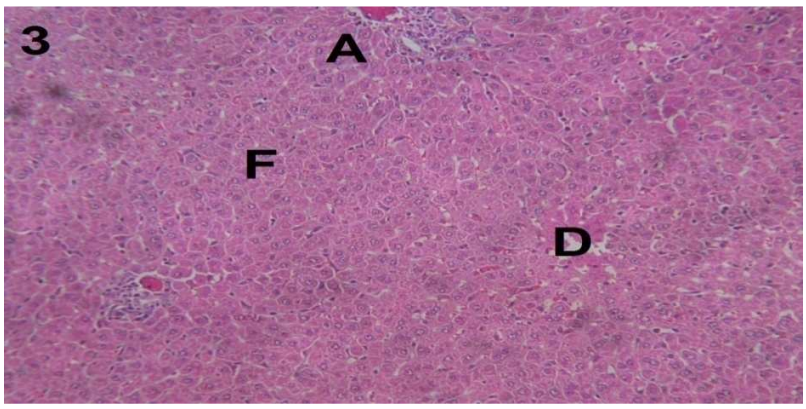


Fig. No. 16. Effect of AEBBL (High) dose on PCM induced hepatic damage

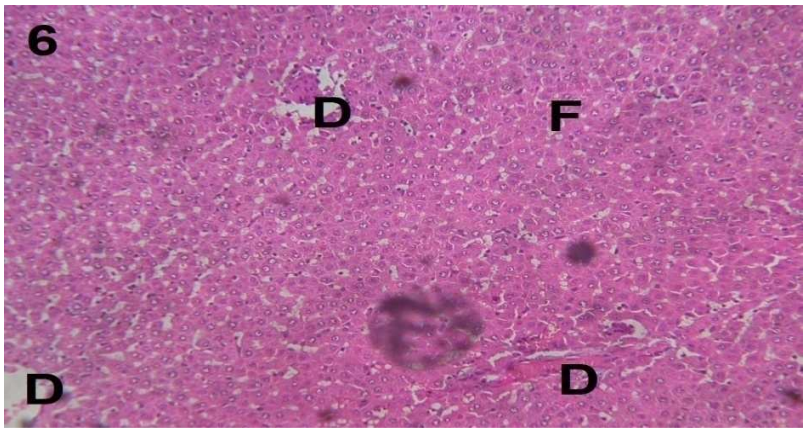
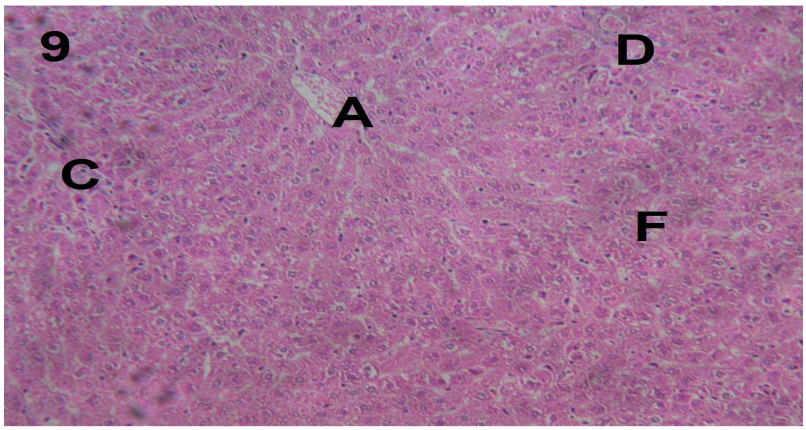


Fig. No. 17. Effect of AQEBBL (High) dose on PCM induced hepatic damage



1: Histology of normal hepatic tissue, 2: PCM induced damage in hepatic tissue, 3: Effect of Silymarin on PCM induced hepatic damage,7: Effect of AQEBBL (Low) dose on ALC induced hepatic damage, 8: Effect of AQEBBL (Med) dose on ALC induced hepatic damage, 9: Effect of AQEBBL (High) dose on ALC induced hepatic damage
A : Central Vein, B : Total Degeneration, C : Sinocoids, D : Portal Traid, E : Portal Inflamation, F : Plates Of Hepatocytes, G : Pknosis

DISCUSSION

The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges, like xenobiotics, drugs, viral infections and chronic alcoholism. If during all such exposures to the above mentioned challenges the natural protective mechanisms of the liver are overpowered, the result is hepatic injury. Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like AST, ALT, triglycerides, cholesterol, bilirubin, alkaline phosphatase are elevated. In spite of phenomenal growth of modern medicine, there are no synthetic drugs available for the treatment of hepatic disorders. However there are several herbs/herbal formulations claimed have possess beneficial activity in treating hepatic disorders. [18]

Paracetamol is normally eliminated mainly as sulfate and glucoronide. Only 5% of the paracetamol is converted into N-acetyl-p-benzoquineimine. However, upon administration of toxic doses of paracetamol the sulfation and glucoronidation routes become saturated and hence, higher percentage of paracetamol molecules are oxidized to highly reactive N-acetyl-p-benzoquineimine (NAPQI) by cytochrome-450 enzymes. Semiquinone radicals, obtained by one electron reduction of NAPQI, can covalently binds to macromolecules of cellular membrane and increases the lipid peroxidation resulting in the tissue damage. Higher dose of paracetamol and NAPQI can alkylate and

oxidize intracellular GSH and protein thiol group, which results in the depletion of liver GSH pool subsequently leads to increased lipid peroxidation and liver damage.[18]

Aspartate and alanine aminotransferases are normally localised within the cells of the liver, heart, gill, kidney, muscles and other organs. The enzymes are of major importance in assessing and monitoring liver cytolysis. Their presence in the serum may give information on organ dysfunction.[19]

ALT is more specific cytosolic enzyme for liver, whereas AST is localized in cytosol and mitochondria that are released into circulation in the early phase of injury. Prolonged destruction in hepatic cells results in more hepatic releases to exacerbate hepatic dysfunction and causes an elevation of ALP, LDH and bilirubin in serum.[20]

Elevated ALP level may indicate cholestasis (partial or full blockade of the bile ducts). Since bile ducts bring bile from the liver into gall bladder and intestine, inflammation/damage of the liver can cause spillage of ALP into the blood stream. ALP and c-glutamyl transpeptidase (GGT) levels typically rise to several times the normal level following bile obstruction or intrahepatic cholestasis. Causes of elevated ALP may also include biliary cirrhosis, fatty liver and liver tumour. The highest serum ALP elevation often greater than 1000 U/l or more than six times the normal value are found in diffuse infiltration of the liver and the biliary tract.[20]

Bilirubin is a yellow pigment produced when heme is catabolized. Hepatocytes render bilirubin water-soluble and therefore easily excretable by conjugating it with glucuronic acid prior to secreting it into bile by active transport. Hyperbilirubinemia may result from the production of more bilirubin than the liver can process, damage to the liver impairing its ability to excrete normal amounts of bilirubin or obstruction of excretory ducts of the liver. Serum bilirubin is considered as one of the true test of liver functions since it reflects the ability of the liver to take up and process bilirubin into bile. Elevated levels may indicate severe illness. High levels of total bilirubin in the acetaminophen induced treated rats may be due to acetaminophen toxicity. This may have resulted in hyperbilirubinemia.[20]

The reduction in the serum and liver levels of albumin in the acetaminophen intoxicated group might be due to liver damage. Hepatotoxicity impairs the synthetic function of the liver. In particular, it reduces albumin production by the liver, and by extension, its serum quantity.[21] Increased in serum CHO and TG level also indicates damage of liver.

In the present study it was observed that Chronic administration of PCM to rats increased the levels of marker enzymes like ALT, AST and ALP as these are stored in the liver cells and increase in the levels of these marker enzymes in serum indicate damage to the liver cells. Pretreatment with AQEBBL decreased the levels of ALT, AST, ALP, BILD, BITD, CHO, TG levels and increased PRO and ALB levels, an indication for the hepatoprotective activity of the extract against drug induced hepatotoxicity.

Intoxication with drugs cause increase in cholesterol and triglyceride levels. AEBBL and AQEBBL prevented elevated cholesterol and triglyceride levels due to hepatic lipid peroxidation occurred after drug intoxication.

In chronic drug induced hepatotoxicity model, administration of thiopentone sodium results with an increased duration of sleeping time, as liver is the primary site for the metabolism of xenobiotics like barbiturates and functional damage to liver requires longer time to inactivate thiopentone resulting with an increased duration of action of this drug. Pretreatment with AEBBL and AQEBBL has decreased the thiopentone induced sleeping time as compared to toxic control indicating their protection of liver function against drug induced toxicity in rats. Liver weight and volume gets increased in toxicant control group. Where in standard and AEBBL and AQEBBL treated groups these were decreased which confirms the hepatoprotective activity of extract.

The protective effect shown by the extracts in functional parameters (Thiopentone induced sleeping time), physical parameters (wet liver weight and wet liver volume), biochemical parameters (ALT, AST & ALP) followed by histological parameters clearly depicts that bark extract of *B. latifolia* possess hepatoprotective activity.

CONCLUSION

➢ Alcoholic (AEBBL) and aqueous (AQEBBL) extracts prepared with bark of *B. latifolia* were evaluated for their hepatoprotective activity in PCM induced hepatotoxic rats.
➢ The preliminary phytochemical analysis of the AEBBL and AQEBBL revealed the presence of tanins, glycosides, saponins, triterpenoids and flavonoids.
➢ L_{D50} studies of both the extracts indicated (Up and Down method) no mortality even up to the highest dose level of 2000 mg/Kg body wt.
➢ Standard reference silymarin has exhibited a significant hepatoprotective activity in PCM, induced hepatoprotoxic model in rats.
➢ From the studies it can be concluded that AEBBL and AQEBBL showed a significant hepatoprotective effect against PCM (curative aspect) induced hepatic damage as depicted by its protective activity on functional, physical, biochemical and histological changes in liver.
➢ The medium and higher doses of AEBBL and AQEBBL (200 and 400 mg/kg) treated groups showed better hepatoprotective activity when compared to standard drug silymarin (100 mg/Kg p.o) treated group.
➢ It is found that the AQEBBL is more potent than AEBBL and that is confirmed by its effect on functional, physical, biochemical parameters followed by comparison of histological changes in liver.
➢ Though both extracts contained similar phytoconstituents, the different in quantity of phytoconstituents present may responsible for the difference in hepatoprotective activity with AQEBBL.

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