

Evaluation of hepatoprotective potential of methanolic extract of the plant *Dolichos biflorus* linn

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Abstract

Present study is to evaluate the hepatoprotective potential of methanolic extract of the plant *Dolichos biflorus* Linn. against paracetamol and alcohol induced hepatotoxicity in rats. Oral administration of plant extract in two doses 200mg/kg and 400mg/kg body weight were subjected for the evaluation of hepatoprotective potential against alcohol (2ml/100g) and PCM (2g/kg) induced liver injury. Silymarin (25mg/kg) was used as a standard drug. The parameters like SGPT, SGO, ALP, TB and endogenous enzymes were estimated to assess the liver functions. In addition histopathological study was also carried out. Both the lower (200mg/kg) and higher dose (400mg/kg) of *D.biflorus* extract showed dose dependent significant decrease in SGPT, SGOT, ALP and TB levels when compared with toxic control. Both extracts showed decrease in LPO and increase in GSH, SOD and CAT levels. Hepatoprotective effect was also confirmed by histopathological analysis of liver which showed less damage in extract treated rats. The results obtained were comparable with that of the standard. The present study concluded that *Dolichos biflorus* Linn. plant were found to be effective against hepatotoxicity induced by Alcohol and Paracetamol.

Keywords: Hepatoprotective; Silymarin; PCM; Alcohol; *Dolichos biflorus*.

1. Introduction

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 ALT, AST, triglycerides, cholesterol, bilirubin and alkaline phosphatase are elevated [1].

It has been estimated that there are some 200 million chronic carriers of the hepatitis B virus of which 40% are expected eventually to die of hepatocellular carcinoma and 15% of cirrhosis. Causative factors of liver disorders include: virus infection, exposure to, or consumption of certain chemicals like chlorinated hydrocarbons, alcohols; medication with antibiotics, chemotherapeutic agents and possibly plant materials such as those containing pyrrolizidine alkaloids; contaminated food containing toxins such as aflatoxins or peroxides in oxidized edible oils; ingestion of industrial pollutants, including radioactive materials[2].

As the liver is so complex, its disorders are equally so, and therefore finding a system for testing plants for the treatment of such disease is not a simple

matter [3]. Thus liver diseases are some of the fatal disease in the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations, which are employed for the treatment of liver disorders [4]. But there is not much drug available for the treatment of liver disorders. In view of this, the present study is undertaken to investigate the hepatoprotective activity of *Dolichos biflorus* plant extract against alcohol and paracetamol induced hepatotoxicity in rats.

2. Materials and Methods

2.1 Collection of the plant material

The *Dolichos biflorus* plant of four month-old was collected from the herbal garden of Mangaluru, Karnataka, India. Authentication of plant is carried out by Mr. Dinesh Nayak, Advisor (Green belt), Mangaluru SEZ Limited India.

2.2 Preparation of Extract

The whole plant was dried in the shade, segregated, pulverized by a mechanical grinder and

passed through a 40 mesh sieve. The powder was extracted by methanol in Soxhlet apparatus by continuous hot percolation method. After filtration through Whatmann filter paper No 40, the filtrate was vacuum dried at 35 to 40°C. The extracts were stored at 40°C until further use. The methanolic extract of *D. biflorus* was subjected to preliminary phytochemical screening to find out the presence of active principles. The collected *Dolichos biflorus* methanolic extract (DBME) was suspended in 50% sucrose solution for oral administration [5].

2.3 Acute Oral Toxicity Study

The methanolic extract of the plant *D. biflorus* was suspended to prepare a dose of 2000mg/kg body weight of animal and administered 1ml/100g body weight of the animal. Acute toxicity study of the extract was done according to acute toxic classic method (OECD guideline 425, 2006) using albino female rats to determine the safe dose. The animals were administered orally with different doses at the limit dose of 2000mg/kg and were observed for 14 days (with special attention for the first 4hr of administration followed by next 20hr) for mortality, general behavior and signs of discomfort. Based on the results obtained from this study, the doses of further pharmacological studies were fixed [6].

2.4 Experimental Animals

Healthy wistar albino rats (100–150g) of either sex were used for the experiment were procured from the animal house of Srinivas College of Pharmacy, Mangaluru. They were maintained under standard conditions (temperature 22±2°C, relative humidity 60±5% and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water ad libitum. The Institutional Animal Ethics Committee approved the experimental protocol (Approval no SCP/CPCSEA/F150/P13/2015). All the animals received human care according to the criteria outlined in the "Guide for the care and use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health". The animals were acclimatized for at least one week before use.

2.5 Drugs and Chemicals

Paracetamol, 40% ethanol, thiobarbituric acid, tris-HCl, potassium dichromate, sodium pyrophosphate, and potassium ferricyanide were purchased from Loba Chemie Lab, Mumbai. Silymarin was from Serum International Ltd, India. NADH, Dithiobinitrobenzoate and Phenazine Methosulphate were purchased from Hemedial Laboratories.

2.6 Paracetamol (PCM) induced liver toxicity[7]

In this experiment, Wistar albino rats were randomly assigned into five groups of six each. The different groups are:

Group I: Vehicle control (50% sucrose, 1 ml/kg)

Group II: Hepatotoxic control (Paracetamol 2g/kg)

Group III: Reference standard (Paracetamol 2g/kg + Silymarin 25mg/kg)

Group IV: *D. biflorus* extract (200mg/kg + Paracetamol 2g/kg)

Group V: *D. biflorus* extract (400mg/kg + Paracetamol 2g/kg)

All the drug preparation and *Dolichos biflorus* methanolic extract (DBME) will be suspended in 50% sucrose solution for oral administration. All the treatment will be given orally once daily throughout the treatment. All the four groups except group I will be intoxicated by oral administration of Paracetamol (2g/kg body weight) on 9th day of treatment. After 48hrs of paracetamol intoxication, blood will be separated and analyzed for various biochemical paracetamol parameters. Animals will be sacrificed by anesthesia; liver will be dissected out and used for histopathological studies.

2.7 Alcohol induced liver toxicity[8]

The Wistar albino rats (150-200g) of either sex were randomly divided into five groups of six each. The different groups were assigned as follows.

Group I: Vehicle control (50% sucrose solution, 1ml/kg)

Group II: Hepatotoxic control (40% v/v alcohol 2ml/100g)

Group III: Reference standard (Silymarin 25mg/kg + 40% v/v alcohol 2ml/100g, p.o)

Group IV: *D. biflorus* plant extract (200mg/kg + 40% v/v alcohol 2ml/100g p.o)

Group V: *D. biflorus* plant extract (400mg/kg + 40% v/v alcohol 2ml/100g p.o)

All the four groups except group I will be intoxicated by 40% v/v ethanol (2ml /100g body weight, p.o) daily for 21 days. All the treatment were given orally once daily for 21 days. After 24hour of ethanol administration on the 22nd day, animals was anesthetized, blood was collected through cardiac puncture and analyzed for various biochemical parameters. Further liver was dissected out and used for histopathological studies. All the biochemical, oxidative stress and histopathological parameters were assessed as per PCM induced hepatotoxicity model.

2.8 Evaluation

2.8.1 Biochemical parameters

a. Estimation of ALP[9]

Serum alkaline phosphatase hydrolyses p-nitrophenyl phosphate into p-nitrophenol and phosphate in the presence of oxidizing agent Mg²⁺.

This reaction is measured as absorbance is proportional to the ALP activity.

b. Estimation of Serum Alanine Transaminase[10]

Alanine amino transferase catalyses the transfer of amino group from alanine to α -keto glutarate (α -KG) with the formation of Glutamate and Pyruvate. The liberated Pyruvate reduced to lactate, by lactate dehydrogenase (LDH) in the same reaction an equivalent amount of NADH is oxidized to NAD.

c. Estimation of Serum Aspartate Amino Transaminase[10]

SGOT catalyses the transfer of amino group from L- Aspartate to α - Keto glutarate with the formation of oxaloacetate and L-glutamate. The rate is monitored by an indicator reaction coupled with malate dehydrogenase (MDH) in which the oxaloacetate formed is converted to malate ion in the presence of NADH (nicotinamide adenine dinucleotide). The oxidation of NADH in this reaction is measured as a decreasing in the absorbance of NADH at 340 nm, which is proportional to SGOT activity.

d. Estimation of Serum Bilirubin[11]

Bilirubin reacts with diazotized sulphanilic acid in acidic medium to form a pink coloured azobilirubin with absorbance directly proportional to bilirubin concentration. Direct bilirubin, being water soluble directly reacted in acidic medium. However, indirect and unconjugated bilirubin is solubilised using a surfactant and then it reacts similar to direct bilirubin.

2.8.2 Estimation of oxidative stress:

a. Lipid peroxidation[12]

Malondialdehyde, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of peroxidation reaction. Malondialdehyde reacts with thiobarbituric acid to form red color species (TBARS), which is measured at 535 nm.

b. Superoxide dismutase (SOD) [13]

The assay of SOD is based on the inhibition of the formation of NADH-phenazine methosulphate-nitroblue tetrazolium formazon. The colour formed at the end of the reaction can be extracted into butanol and measured at 560nm.

c. Reduced glutathione (GSH)[14]

Reduced glutathione on reaction with DTNB (5, 5'-dithiobis nitro benzoic acid) produces a yellow coloured product that absorbs at 412nm.

d. Catalase (CAT) [15]

The liver was homogenized in 0.01 M phosphate buffer (pH 7.0) and centrifuge at 5000 rpm. The reaction mixture consisted of 0.4 ml of hydrogen peroxide (0.2 M), 1 ml of 0.01 M phosphate buffer (pH 7.0) and 0.1 ml of liver homogenate (10% w/v).

The reaction of the mixture was stopped by adding 2 ml of dichromate-acetic acid reagent (5% K₂Cr₂O₇ prepared in glacial acetic acid). The changes in the absorbance was measured at 620 nm and recorded. Percentage inhibition was calculated using the equation:

$$\% \text{ Increase in CAT} = \frac{(\text{Abs of control} - \text{Abs of test})}{\text{Abs of control}} \times 100$$

2.9 Histopathological studies

The livers were removed from the animals and the tissues were stored in 10% formalin. The liver samples were sent for histopathological studies.

2.10 Statistical analysis

All data were expressed as mean \pm SEM. The statistical significance between groups was compared using one way ANOVA, followed by Dunnett's (multiple comparisons) test.

3. Results

3.1 Hepatoprotective activity of *D.biflorus* on PCM induced hepatic damage in rats

Administration of paracetamol to rats caused significant liver damage, as evidenced by the altered serum biochemical parameters. Pre-treatment of rats with *Dolichos biflorus* plant extract exhibited marked protection against paracetamol induced hepatotoxicity. The effects produced by *Dolichos biflorus* plant extract were comparable with that produced by the standard; Silymarin. The results were further confirmed by evaluation of liver endogenous antioxidant enzymes of the rats.

The effect of plant extracts of *D.biflorus* on various biochemical parameters are shown in Table No.1 and Fig.1 and 2.

Group I (Control): The SGPT, SGOT, ALP and Total Bilirubin levels were 89.0 ± 1.15 , 110.3 ± 1.35 , 141.0 ± 2.01 and 0.73 ± 0.02 respectively. Histopathological study showed normal portal triad, sinusoids and cord arrangement of hepatocytes. (Figure no: 4A)

Group II: The paracetamol induced group showed elevation in SGPT, SGOT, ALP and Total Bilirubin levels up to 288.3 ± 1.47 , 382.7 ± 3.38 , 388 ± 2.49 and 2.23 ± 0.02 respectively when compared to the control group. Histopathological study showed portal tract inflammation. (Figure no: 4B)

Group III: There was a significant ($p < 0.05$) reduction in the SGPT, SGOT, ALP and Total Bilirubin levels (97.83 ± 1.66 , 123.8 ± 2.71 , 152.3 ± 2.12 and 0.85 ± 0.02 respectively) after the treatment with Silymarin (100mg/kg). Histopathological study showed almost normal appearing hepatocytes. (Figure no: 4C)

Group IV: There was a reduction in the SGPT, SGOT, ALP and Total bilirubin levels (195.2 ± 1.13 ,

285.2 ± 1.30, 294.1 ± 1.27, 1.74 ± 0.01) after the treatment with 2ml/kg of *Dolichos biflorus* plant extract. Histopathological study showed mild portal tract inflammation. (Figure no: 4D)

Group V: There was a significant (p<0.05) reduction in the SGPT, SGOT, ALP and Total bilirubin levels

(134.5 ± 1.12, 182.5 ± 0.76, 192.0 ± 1.18 and 1.15 ± 0.01 respectively) after the treatment with 4ml/kg *Dolichos biflorus* plant extract. Histopathological study showed minimal portal tract inflammation. (Figure no: 4E)

Table 1: Effect of Silymarin and *D.biflorus* on SGOT, SGPT, ALP and Total Bilirubin in PCM induced liver toxicity.

Groups	Treatment	ALP (U/I)	SGOT (U/I)	SGPT (U/I)	TB (mg/dl)
Vehicle control	50% sucrose 1ml/ Kg	141.0±2.01	110.03±1.35	89.0±1.15	0.73±0.02
Toxic control	PCM 2g/Kg p.o	388.1±2.49 ^a	382.7±3.38 ^a	288.3±1.47 ^a	2.23±0.02 ^a
Standard	Silymarin 25mg/Kg, p.o	152.3±2.12 ^{***}	123.8±2.71 ^{***}	97.83±1.66 ^{***}	0.85±0.02 ^{***}
Low dose	<i>D.biflorus</i> 200mg/Kg, p.o	294.1±1.27 [*]	285.2±1.30 [*]	195.2±1.13 [*]	1.74±0.01 [*]
High dose	<i>D.biflorus</i> 400mg/Kg, p.o	192.0±1.18 ^{**}	182.5±0.76 ^{**}	134.5±1.12 ^{**}	1.15±0.01 ^{**}

All the values are Mean±SEM, n=6. One way ANOVA followed by Dunnett's t test. ^ap<0.001 when compared with vehicle treated control group. *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

Figure 1: Effect of Silymarin and *D.biflorus* on Serum SGPT, SGOT& ALP in PCM induced liver toxicity

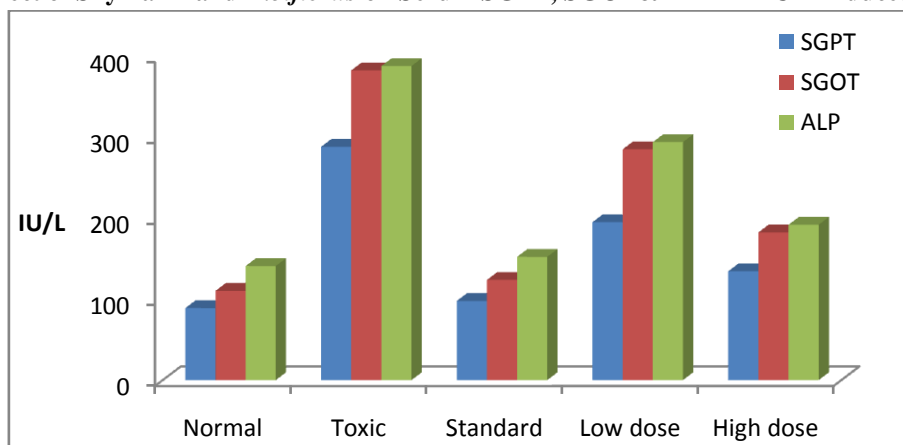
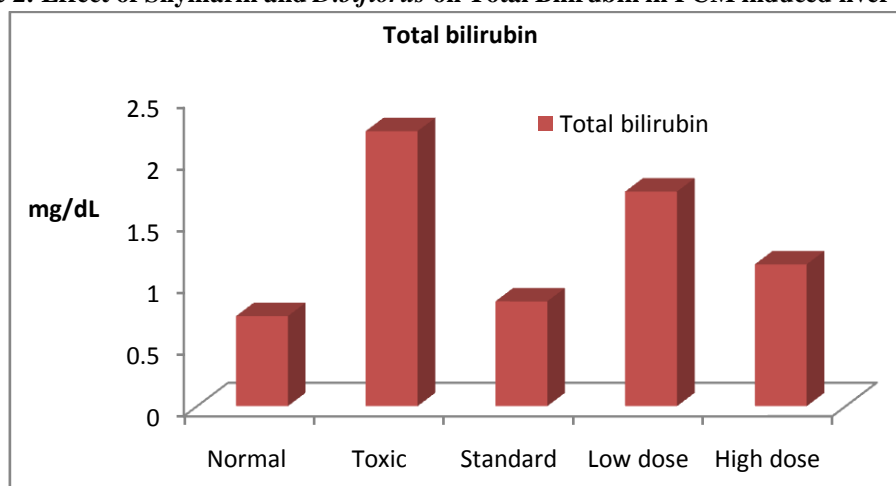


Figure 2: Effect of Silymarin and *D.biflorus* on Total Bilirubin in PCM induced liver toxicity.



3.2 Evaluation of liver endogenous antioxidant enzymes

Table No.2 and Fig.3 shows the effects of extracts of *D.biflorus* on LPO, SOD, GSH and CAT concentrations in rat liver after challenging with paracetamol. It was observed that animals treated with PCM developed a hepatic damage, increase in LPO and decrease in GSH, CAT & SOD when compared to normal control. Animals treated with standard

(Silymarin) showed extremely significant (P<0.001) increase in GSH, CAT & SOD and decrease in LPO. *D.biflorus* (200mg/kg) treated animals showed significant (P<0.05) decrease in LPO and significant (P<0.05) increase in GSH, CAT & SOD. *D.biflorus* (400mg/kg) treated animals showed moderately significant (P<0.01) decrease in LPO and moderately significant (P<0.01) increase in GSH, SOD & CAT.

Table 2: Effect of Silymarin and *D.biflorus* on LPO, SOD, GSH, and CAT in PCM induced liver toxicity

Groups	Treatment	LPO (Abs at 535 nm)	SOD (Abs at 560 nm)	GSH (Abs at 412nm)	CAT (Abs at 620 nm)
Normal Control	50% Sucrose 1ml/Kg	2.96± 0.89	21.81±1.25	28.0 ± 1.01	43.1 2± 0.971
Toxic control	PCM 2g/Kg p.o.	16.71± 3.23 ^a	13.18±3.12 ^a	18.19 ± 4.14 ^a	27.15 ± 4.95 ^a
Standard	Silymarin 25mg/Kg p.o.	5.31± 1.41 ^{***}	20.22±2.59 ^{***}	23.15 ± 1.81 ^{***}	39.4 3± 2.04 ^{***}
Low dose	<i>D.biflorus</i> 200mg/Kg p.o.	11.12± 2.21 [*]	14.56±3.81 [*]	19.3 0 ± 2.14 [*]	32.01±3.01 [*]
High dose	<i>D.biflorus</i> 400mg/Kg p.o.	8.12± 1.19 ^{**}	16.20±2.65 ^{**}	21.1 4±1.81 ^{**}	35.33 ± 3.51 ^{**}

All the values are Mean±SEM, n=6. One way ANOVA followed by Dunnett's t test, ^ap<0.001 when compared with vehicle treated control group. *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

Figure 3: Effect of Silymarin and *Dolichos biflorus* on SOD, GSH, CAT, and LPO in PCM induced liver toxicity

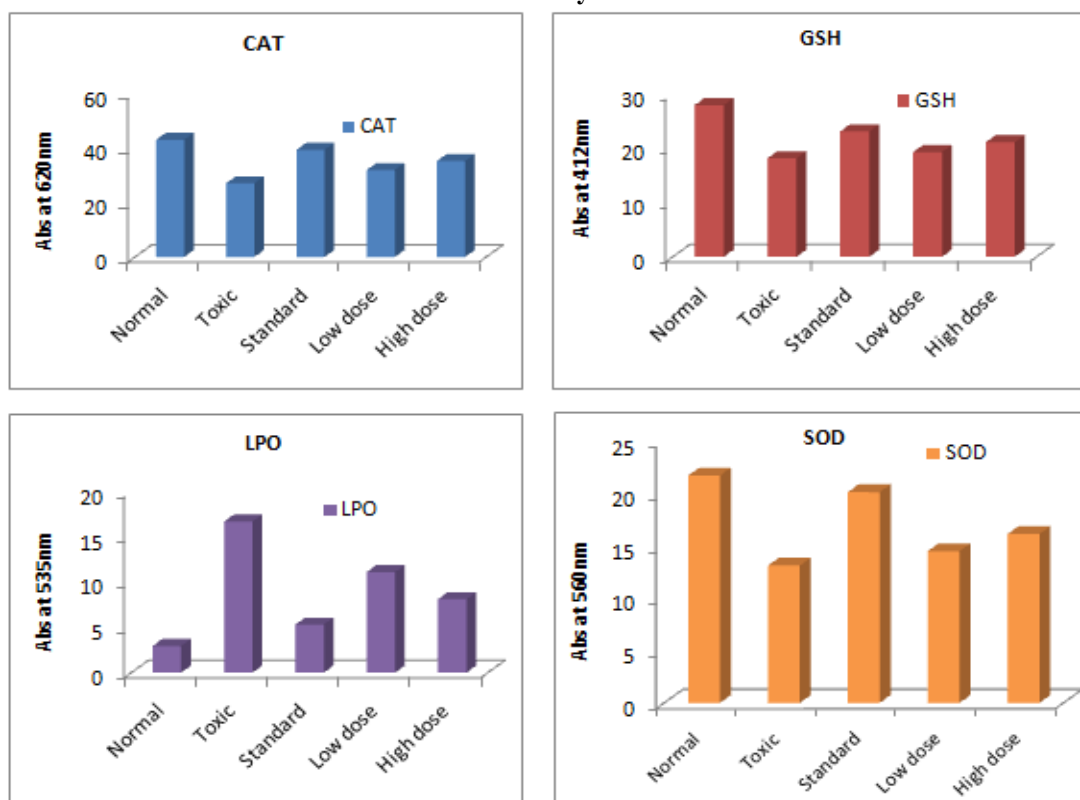
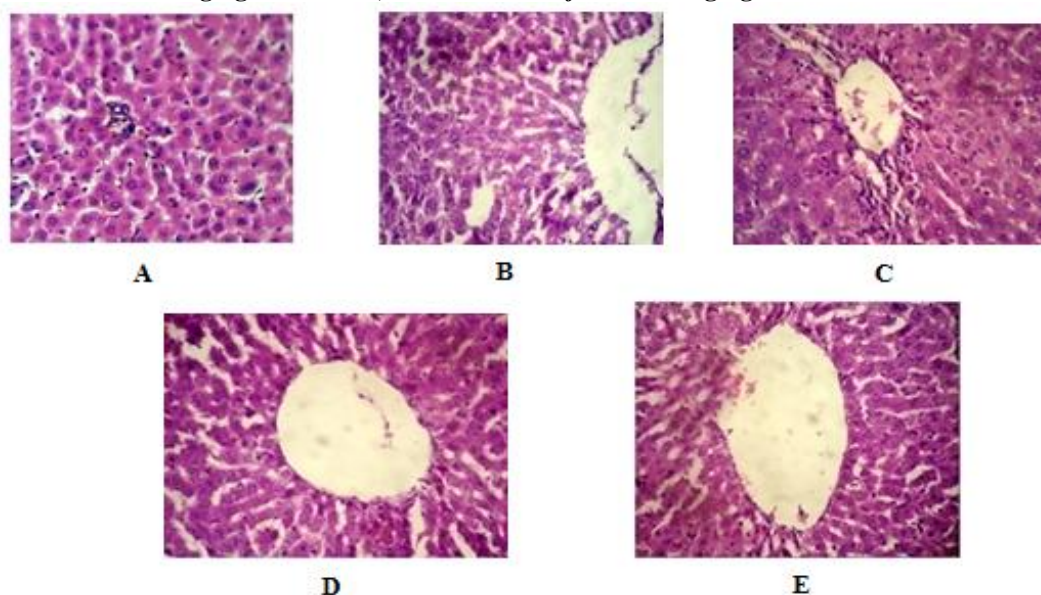


Fig.4: Effect of Silymarin and *D.biflorus* on liver histology in PCM induced liver toxicity. A: Liver of normal rat; B: Liver of PCM induced rat; C: Liver of Silymarin treated rat; D: Liver of *D.biflorus* 200 mg/kg treated rat; E: Liver of *D.biflorus* 400mg/kg treated rat.



3.3 Hepatoprotective activity of *D.biflorus* on alcohol induced hepatic damage in rats

In the present study, the hepatotoxicity was successfully produced by administration of alcohol 2ml/100g body weight p.o for once daily and the hepatoprotective activity of *D.biflorus* was determined from the serum parameters SGOT, SGPT, ALP and TB. The results were further confirmed by evaluation of liver endogenous antioxidant enzymes.

The effect of methanolic extract of *Dolichos biflorus* plants on various biochemical parameters are shown in Table No.3 and Fig 5. It was observed that, the activities of serum SGOT, SGPT, ALP & TB were

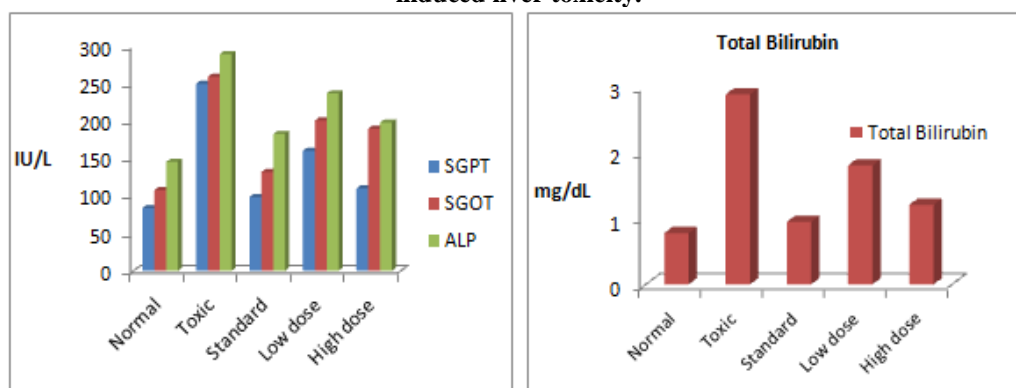
increased markedly in alcohol fed animals as compared to normal control group. The administration of *Dolichos biflorus* extract 200mg/kg and 400mg/kg lowered the alcohol induced elevation of serum parameters. The Standard (Silymarin) treatment showed extremely significant (P<0.001) reduction in SGPT, SGOT, ALP, and TB. *Dolichos biflorus* (200mg/kg) treated animals showed significant (P<0.05) reduction in SGPT, SGOT, ALP & TB levels as compared to toxic control group. *Dolichos biflorus* extract 400mg/kg treated animals showed moderately significant (P<0.01) reduction in SGOT, SGPT, ALP & TB level as compared to toxic control group.

Table 3: Effect of Silymarin and *Dolichos biflorus* on SGOT, SGPT, ALP & TB in alcohol induced liver toxicity.

Groups	Treatment	ALP(U/I)	SGOT(U/I)	SGPT(U/I)	TB(mg/dl)
Normal control	50% sucrose solution	145.7± 2.12	1080.01± 1.16	83.9± 1.28	0.78± 0.08
Toxic control	Alcohol 2ml/100 g p.o	289.91± 2.05	259.51± 1.59	250.01± 1.61	2.89± 0.12
Standard	Silymarin 25mg/Kg, p.o	183.0±2.17***	132.1±2.13***	98.83±1.51***	0.95±0.01***
Low dose	<i>Dolichos biflorus</i> 200mg/Kg, p.o	237.11± 1.67*	201.09± 1.97*	160.5± 2.09*	1.81± 0.02*
High dose	<i>Dolichos biflorus</i> 400mg/Kg, p.o	198.48±1.16**	190.31±1.48**	110.21±1.21**	1.21±0.03**

All the values are Mean±SEM, n=6. One way ANOVA followed by Dunnett's t test. ^ap< 0.001 when compared with vehicle treated control group. *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

Figure 5: Effect of Silymarin and *Dolichos biflorus* on serum SGPT, SGOT, ALP and TB in alcohol induced liver toxicity.



3.4 Evaluation of liver endogenous antioxidant enzymes

Table No.4 and Graphs Fig.6 shows the effects of extracts of *Dolichos biflorus* on SOD, CAT, GSH and lipid peroxidation concentrations in rat liver after challenging with alcohol. It was observed that animals treated with alcohol developed a hepatic damage observed as increase in LPO and decrease in GSH, CAT & SOD when compared to normal control. Animals treated with standard (Silymarin) showed

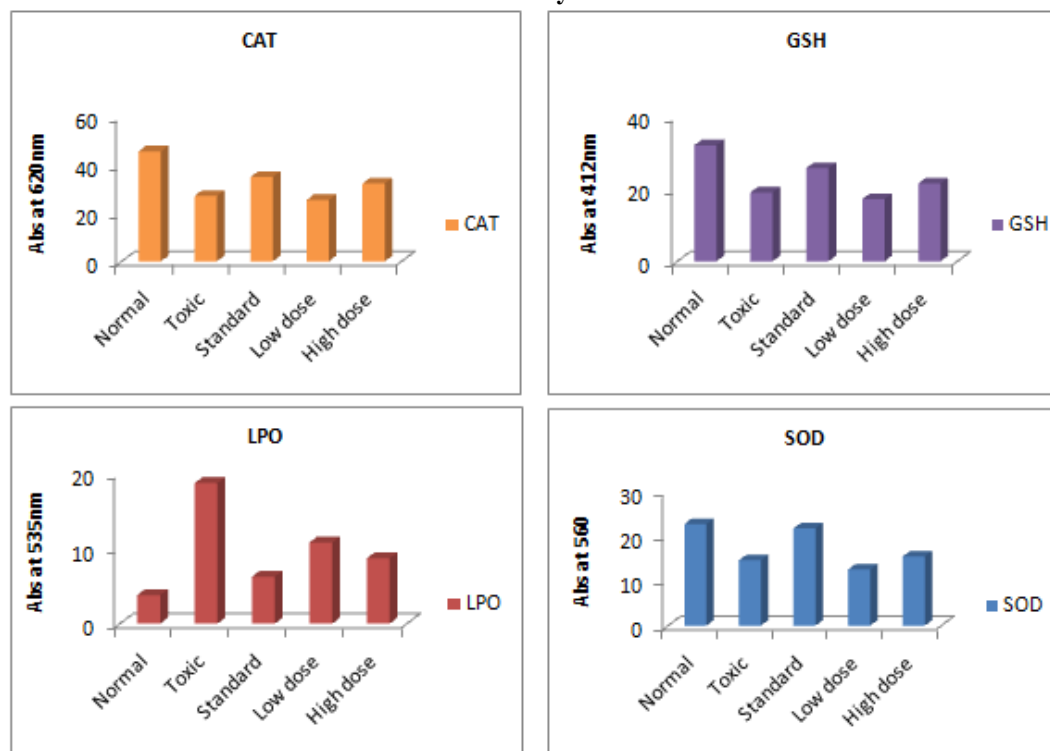
extremely significant (P<0.001) increase in GSH, CAT & SOD and decrease in LPO. *Dolichos biflorus* extract (200mg/kg) treated animals showed significant (P<0.05) decrease in LPO and significant (P<0.05) increase in SOD, GSH & CAT as compared to toxic control. *Dolichos biflorus* extract (400mg/kg) treated animals showed moderately significant (P<0.01) decrease in LPO and moderately significant (P<0.01) increase in GSH, SOD & CAT.

Table 4: Effect of Silymarin and *Dolichos biflorus* on LPO, SOD, GSH, and CAT in alcohol induced liver toxicity

Groups	Treatment	LPO (Abs at 535 nm)	SOD (Abs at 560 nm)	GSH (Abs at 412nm)	CAT (Abs at 620 nm)
Normal Control	50% sucrose solution	3.81± 0.90	23.12± 2021	32.15± 1.12	45.71± 0.99
Toxic control	Alcohol 2.0ml/100 g	18.81± 4.01 ^a	14.91± 3.92 ^a	19.06± 4.01 ^a	27.14± 3.89 ^a
Standard	Silymarin 25mg/kg	6.28± 1058***	22.15± 2.31***	25.81± 1.56***	35.01± 2.11***
Low dose	<i>Dolichos biflorus</i> 200mg/kg	10.86± 2.38*	12.81± 3.23*	17.21± 2.45*	25.51± 3.34*
High dose	<i>Dolichos biflorus</i> 400mg/kg	8.73± 1.01**	15.78± 2.19**	21.54± 1.78**	32.3± 3.61**

All the values are Mean±SEM, n=6. One way ANOVA followed by Dunnett's t test. ^ap< 0.001 when compared with vehicle treated control group. *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

Figure 6: Effect of Silymarin and *Dolichos biflorus* on SOD, GSH, CAT, and LPO in alcohol induced liver toxicity.

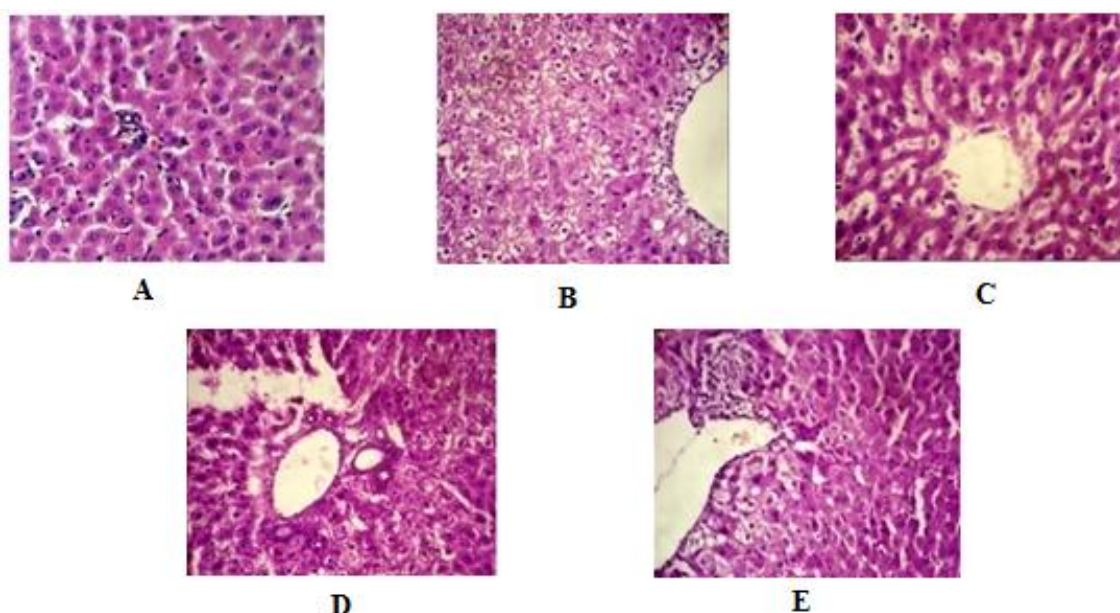


3.5 Histopathological studies of the liver

The histopathological evaluation of Alcohol toxicity in all the groups was examined and shown in Fig. 7. Liver section of normal group shows liver parenchyma with intact architecture. Most hepatocytes appear normal. In toxic control group shows inflammation, centrilobular degeneration and

necrosis. Treatment with *Dolichos biflorus* (200mg/kg & 400mg/kg) found to reduce inflammation, centrilobular and bridging necrosis. Liver section of this group shows normal hepatocytes with significant reduction in areas of necrosis when compared to toxic control group. These changes show protective effect of the drug against hepatic damage induced by alcohol.

Fig. 7: Effect of Silymarin and *Dolichos biflorus* on liver histology in alcohol induced liver toxicity.



A: Liver of normal rat; **B:** Liver of alcohol induced rat; **C:** Liver of Silymarin treated rat; **D:** Liver of *Dolichos biflorus* 200mg/kg treated rat; **E:** Liver of *Dolichos biflorus* 400mg/kg treated rat.

4. Discussions

The present study was undertaken to evaluate the hepatoprotective activity of *Dolichos biflorus* plant extract. The study was conducted by using two models i.e., paracetamol alcohol induced liver damage model. The parameters used for the assessment of hepatoprotective activity were serum enzyme estimations like SGPT, SGOT, ALP, Total bilirubin and reduced serum CAT, SOD and GSH level and histopathological studies.

4.1 Hepatoprotective activity of *Dolichos biflorus* plant extract on paracetamol induced liver damage

Administration of paracetamol elevated the serum levels of SGPT, SGOT, ALP and Total bilirubin significantly. This is due to its bioactivation to a toxic electrophile, N-acetyl-p-benzoquinone-imine. Paracetamol is normally eliminated mainly as sulphate and glucuronide. Only 5% of the paracetamol is converted into N-acetyl-p-benzoquinoneimine. However, upon administration of toxic doses of paracetamol, the sulfation and glucuronidation routes become saturated and hence, higher percentage of paracetamol molecules are oxidized to highly reactive N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome-450 enzymes. A semi Quinone radical, obtained by one electron reduction of NAPQI, can covalently bind to macromolecules of cellular membrane and increases the lipid peroxidation resulting in the tissue damage.

The hepatotoxic effect of paracetamol was also confirmed by histopathological studies which showed portal tract inflammation. Pre-treatment with *Dolichos biflorus* plant extract was able to prevent the elevation of SGPT, SGOT, ALP and Total bilirubin by paracetamol. These biochemical effects may be due to the inhibitory effects on cytochrome P450 and/or promotion of its glucuronidation. This was also confirmed by histopathological studies which showed almost normal hepatocytes.

4.2 Hepatoprotective activity of *Dolichos biflorus* plant extract on alcohol induced liver damage:

Alcohol consumption is known to cause fatty infiltration, hepatitis and cirrhosis. Fat infiltration is a reversible phenomenon that occurs when alcohol replaces fatty acids in the mitochondria. Hepatitis and cirrhosis may occur because of enhanced lipid peroxidative reaction during the microsomal metabolism of ethanol. It is generally accepted that alcohol can induce *in vivo* changes in membrane lipid composition and fluidity, which may eventually affect cellular functions. Among the mechanisms responsible for effects of alcohol, an increase in hepatic lipid peroxidation leads to alteration in membrane phospholipid composition. The effects of ethanol have been suggested to be a result of the enhanced generation of free radicals during its oxidation in liver.

The peroxidation of membrane lipid results in loss of membrane structure and integrity. This result in elevated levels of membrane bound enzymes in serum. Ethanol inhibits glutathione peroxidase; decrease the activity of catalase, superoxide dismutase, along with increase in levels of glutathione in liver. The decrease in activity of antioxidant enzymes like superoxide dismutase, glutathione peroxidase are speculated to be due to the damaging effects of free radicals produced following ethanol exposure or alternatively could be due to a direct effect of acetaldehyde, formed by oxidation of ethanol. The plant extract *D.biflorus* (200mg/kg and 400mg/kg) showed dose dependent decrease in the elevated serum biomarkers (SGPT, SGOT, ALP and bilirubin), lipid peroxidation, and significant increase in endogenous enzymes (GSH, SOD, CAT). Hence it might be possible that the mechanism of hepatoprotection by *D.biflorus* plant is due to its antioxidant potential.

Histopathological studies of liver, treated with alcohol alone revealed the affected architecture of liver parenchyma with damaged hepatocytes. Treatment with *D.biflorus* (200mg/kg & 400 mg/kg) revealed significant improvement in architecture of liver parenchyma towards normal and regenerating hepatocytes indicating hepatoprotection. The standard drug Silymarin showed extremely significant reduction in serum biomarkers and endogenous enzyme level. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many plant extracts. Natural antioxidants from the plant extracts provide a measure of production of radical scavengers that slows the process of oxidative damage; further previous report indicates that the *D.biflorus* plant extract proved for its potential antioxidant properties. The present study revealed that the *D.biflorus* plant extract have proved its synergistic antioxidant effects of bioactive constituents for observed hepatoprotective activity.

5. Conclusions

The present study was undertaken to assess the hepatoprotective activity of *Dolichos biflorus* plant extract. The extract found to have significant hepatoprotective activity in both; paracetamol and alcohol induced hepatic injury models. Biochemical and histopathological studies have revealed that this plant have comparable hepatoprotective activity with that of Silymarin. It leads to the conclusion that the *Dolichos biflorus* plant extract can be utilized for its hepatoprotective activity. Further studies are needed to isolate and characterize the active principles and to find out the mechanism responsible for its hepatoprotective activity.

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