Evaluation Of Hepatoprotective Activity Of Ethanol Extract Of Dioscorea Bulbifera L. On Ccl_4 Induced Hepatotoxicity In Experimental Rats

Shaik Ayesha, S. Kalpana*, S Nelson Kumar, R. Manohar and C Rajaram

Department of Pharmacology, P. Rami Reddy Memorial College of Pharmacy, Kadapa - 516 003, Andhra Pradesh, India.

***Corresponding author:**

Mrs. S. Kalpana

kalpanasekhar135@gmail.com

ABSTRACT

OBJECTIVE: The present study is to evaluate hepatoprotective activity of ethanol extract of *Dioscorea bulbifera* L. on CCl4 Induced liver damage on experimental rats.

METHODS: The ethanol extract of *Dioscorea bulbifera* L. (Dioscoreaceae) was investigated for CCl4 Induced hepatotoxicity. Silymarin was used as a reference drug at a dose of 50 mg/kg b.w. All the experimental animals were divided into 5 groups of 6 each. In this study, hepatoprotective effect of the two different doses (200 and 400 mg/kg b.w. p.o) of EEDB will be investigated for 28 days to evaluate dose dependent activity. Effect of EEDB on biochemical parameters, liver homogenate enzymes and also for histopathological studies.

RESULTS: All the values show significant activity over hepatoprotective activity of EEDB on consumption of the extracts, with greater effect exhibited by the 400 mg/kg extract. At the end of the experiment the results of biochemical analysis and histopathological studies show significant changes on compared with disease control group.

CONCLUSION: From the results, it was concluded that EEDB have hepatoprotective activity. Further studies will be needed in future in order to determine which one or more of its active constituents have the main effects.

Key Words: CCl4, Hepatoprotective activity, *Dioscorea bulbifera* L.

DOI: 10.48047/ecb/2023.12.si12.006

INTRODUCTION

The liver is the largest gland of the body enclosed within the right lower rib cage beneath the diaphragm. Liver is the crucial organ for maintenance of gastrointestinal homeostasis and normal body functions by its strategic location and multidimensional functions to support other body functions. In addition to its role in the digestive process, it also serves as a source of nutrients and detoxifier of unwanted substances by its metabolising function. Hence, its optimal functioning is crucial for health and disease [1]. Hepatotoxicity implies to liver dysfunction or liver damage that is caused by overload of drugs or xenobiotic [2]. Certain drugs once taken in overdoses and generally even when introduced inside the therapeutic ranges, may injure the organs [3]. The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents.

Chemical-induced liver damage is referred to as hepatotoxicity. Overdosing on certain medications, and sometimes even introducing them within therapeutic limits, can harm the organ. Other synthetic specialists, like those utilized in research facilities and enterprises, regular synthetic compounds (e.g., microcystins) and herbal remedies can also induce hepatotoxicity [4]. Chemicals that cause liver injury are called hepatotoxins. Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures. More than 75 percent of cases of idiosyncratic drug reactions result in liver transplantation or death [5].

Dietary habits, alcohol consumption, poor hygiene, unsupervised drug use, smoking, and other factors all contribute to liver disorders, which are the most prevalent health risk in developing nations. Liver sicknesses can be non-inflammatory, inflammatory and degenerative. Elevated levels of plasma total cholesterol (LDL-C) and triacylglycerols (TGs) are related with high risk of atherosclerosis and cardiovascular illness inferable from the hepatic inadequacy [6 & 7]. Hepatotoxicity caused by many toxins carbon tetrachloride (CCl4), thioacetamide, acute or chronic alcohol consumption, various infections like hepatitis A, B, C and drugs, in which drugs are most common offender. Free radical generations in the alcohol use result in development of hepatitis leading to cirrhosis [8].

India is rightfully referred to as the "Botanical Garden of the World" because it is the largest producer of medicinal plants. The medicinal plants have vital spot in the wellbeing and imperativeness of people as well as animals. According to the WHO gauges, around 3/4 of the total population right now use herbs and other conventional drugs to fix different sicknesses,

including liver diseases. As a result, a number of phytomedicines - herbal or medicinal plants are now used to treat and prevent a variety of liver disorders [9].

Dioscorea bulbifera L. is a tribal plant belonging to the family of Dioscoreaceae. It is a climber plant with tuberous root. Dioscorea is a large genus of annual twining herbs, distributed throughout the moist tropics of world and extending into warm temperate regions. About 50 species are found in India [10]. The plant is proved for anticancer, anti-inflammatory, analgesic, antidiabetic, anti HIV, diuretic and antioxidant activity [11]. Still there was lack in scientific study for hepatoprotective activity of *Dioscorea bulbifera* L. to substantiate the traditional claim. Hence, the current work was embraced to assess the hepatoprotective activity of ethanol extract of *Dioscorea bulbifera* L.

MATERIALS AND METHODS

Chemicals and reagent

All the chemicals and reagents used in these studies were of analytical grade.

Collection and authentication of plant material

Fresh leafs of *Dioscorea bulbifera* L. (Dioscoreaceae) were pull together from surrounded arears of kadapa region and authentified by Dr. K. Madava Chetty, Professor, Department of Botany, Sri Venkateswara University, Tirupathi. Andhra Pradesh, India.

Preparation of plant material

The gathered leafs of *Dioscorea bulbifera* L. was washed with running water, cut into little pieces and shade dried at room temperature to avoid from loss of phytoconstituents of plant. The total shade dried materials pounded for powder and sieved up to 80 meshes. At that point it was homogenized to fine powder and put away in air-tight compartment for further activities.

Preparation of plant extracts

Powder of the *Dioscorea bulbifera* L. was extracted with ethanol as a solvent in a Soxhlet apparatus in batches of 500 gm each. The excess solvent was expelled from extract utilizing a rotary vacuum evaporator and later on concentrated on a water bath. At last dried extracts were put away in desiccators for assess for its antidiabetic activities [12].

Animals and maintenance

Wistar Albino rats of either sex, with the body weight of 150-250 gms were acquired from Sri Venkateswara Enterprises, Bangalore, India. Animals were kept up according to rules of NIN animal client manual. Animals are adjusted for 10 days to our creature house, kept up at temperature of 22ºC to ± 2 ºC. The animal was directed by a 12 hours light, 12 hours dark calendar. Five animals are housed per cage estimated 41 cm length, 28 cm width and height of

14 cm. Paddy husk was utilized for bedding and on elective day bedding was changed and washed altogether with water using domex, a disinfectant and detergenic. The rats were feed with a standard pellet diet bought from Suresh organizations, Hyderabad and water not obligatory. The examination convention was investigated and endorsed by the Institutional Animal Ethical Committee (IAEC) and trials were done according to the rules of CPCSEA. Reg. Number: 1423/PO/Re/S/11/CPCSEA, date 25th November 2022.

Experimental design

The experimental design used to carry out the hepatoprotective activity of ethanol extract of *Dioscorea bulbifera* L. (EEDB) against CCl_4 induced hepatic damage model. The animals were grouped into 5 groups with 6 animals in each. Except the normal group all the other groups received carbon tetrachloride (CCl_4) 50% v/v in coconut oil at a dose of 0.1 ml/kg b.w intraperitoneally for 28 days. Normal groups received plain coconut oil orally. The standard group received silymarin 50 mg/kg orally. Test groups received EEDB 200 mg/kg and 400mg/kg b.w orally. On the 28th day, blood was collected from each animal for serum analysis. The rats were sacrificed, and their livers were removed. One lobe was then fixed in 10% formalin for histopathological studies and the remaining part was subjected for antioxidant study [13].

Group – I - Normal control receive 1% CMC.

Group – II - Disease control receive CCl_4 in coconut oil (0.1ml / kg b. w., i.p).

Group – III - Standard, received CCl_4 in coconut oil (0.1ml / kg b. w., i.p) + Silymarin (50 mg/kg b. w.).

Group – IV - Test, Received CCl_4 in coconut oil (0.1ml / kg b. w., i.p) + EEDB (200 mg/kg b. w.).

Group – V - Test, Received CCl_4 in coconut oil (0.1ml / kg b. w., i.p) + EEDB (400 mg/kg b. w.).

Assessment of serum marker enzymes

The blood samples were collected in EDTA-free vials on day 28th. The collected blood samples were centrifuged under cooling condition at 4000 RPM for 10-15 minutes to separate plasma and serum. The separated serum was used for the estimation of biochemical parameters like SGOT, SGPT, ALP, total cholesterol, total albumin, total protein and TB using commercial available diagnostic kit (Span Diagnostic, Ltd) [14].

Assessment of pro-oxidant and antioxidant enzymes:

Estimation of catalase (CAT) [15]:

The Hugo E. Aebi method was used for the estimation of catalase: hydrogen peroxide: hydrogen- peroxidoreductase.

Reagents:

1. Phosphate buffer (50Mm, p^H 7.0)
2. Hydrogen peroxide (30 Mm/I)

Procedure:

Dilute homogenate 20 times with buffer p^H 7.0.

Blank	Test
4ml of homogenate diluted with 2ml of phosphate buffer p^H 7.0, and take absorbance at 240 nm for 3min with 30 sec intervals.	2ml of homogenate diluted with 1ml of H_2O_2 (8.5 micro lit. in 2.5 ml) phosphate buffer (50Mm, p^H 7) and take the absorbance at 240nm for 3 min with 30 sec intervals. (Add H_2O_2 just before taking O.D)

Log (A/B) × 2297.3

Where,

A: Initial abdsorbance.

B: Final absorbance (after 30 sec)

Units = µ moles of H_2O_2 consumed/min/mg.

Estimation of reduced glutathione (Reduced GSH) [16]:

Reagents:

1. **TCA (10% W/V) solution:** Accurately weighed 10g of TCA was dissolved in 100 ml of distilled water.
2. **Phosphate buffer** (0.2 M, p^H 8).
3. **DTNB reagent (0.6 M):** 60mg of 5,5-dithio bis (2-nitrobenzoic acid) was dissolved in 100ml of 0.2M sodium phosphate (p^H 8).
4. **Standard glutathione:** Prepared by dissolving 10mg of reduced glutathione in 100 ml of distilled water.

Procedure:

1. 1ml of 10% TCA was added to 1ml of sample.
2. The precipitated fraction was centrifuged and to 0.5ml of supernatant, 2ml of DTNB was added.
3. The final volume was made up to 3ml with phosphate buffer.

4. The colour developed was read at 412 nm.
5. The amount of glutathione was expressed as µg of GSH mg of protein.
6. Reduced glutathione was used as standard (100 µg/ml).

$$\mathbf{X}= \frac{(\boldsymbol{Y}-\mathbf{0.0046})}{\mathbf{0.0034}}$$

Y= Absorbance of test sample.

Estimation of pro-oxidant (MDA) [17]:

Reagents:

1. **Thiobarbituric acid (0.67% W/V):** In 1ml tris hydrochloride p^H -7, 0.67 g of thiobarbituric acid was dissolved in 100ml of distilled water.
2. **Trichloroacetic acid (20% w/v):** 20g of TCA was dissolved in 100 ml of distilled water.
3. **Standard malondialdehyde** (0-25 n.mol)

Procedure:

1. 2ml of sample was mixed with 2ml of 20% TCA and kept in ice for 15 min.
2. The precipitate was separated by centrifugation and 2ml of samples of clear supernatant solution were mixed with 2ml of aq. 0.67% TBA solution.
3. This mixture was heated on a boiling water bath for 10 min.
4. It was cooled and absorbance was read at 535 nm.
5. The values are expressed as nm of MDA formed /mg of protein values are normalized to protein content of tissues.

$$\mathbf{X} = \frac{\mathbf{Y} + \mathbf{0.002}}{\mathbf{0.0026086}}$$

Y: Absorbance difference of final (after 3 min) and initial reading of test sample.

Estimation of super oxide dismutase (SOD) [18]:

Reagents:

1. **EDTA** **:** (1×10^{-4}M)
2. **Epinephrine** **:** (3×10^{-3}M)
3. **Carbonate buffer** **:** (p^H=9.7)

Procedure:

1. 0.1ml of sample was dissolved in 0.1ml of EDTA followed by the addition of 0.5ml of carbonate buffer and 1ml of epinephrine.
2. The optical density of formed adrenochrome was measured at 480 nm for 3 min at an interval of 30 sec.

3. Results were expressed as mUnits/mg protein.

One unit of enzyme activity is defined as the enzyme concentration required to inhibit the chromogen production by 50% in one hour under the defined assay conditions.

Histopathological studies:

On day 28th, one representative animal from each group was utilized for this purpose. The experimental animals were sacrificed under mild ether sedation to collect the liver. The liver specimens obtained from the control and treated groups of animals were fixed in 10 % buffered formalin for 24 h. The formalin fixed liver samples were stained with haematoxylin-eosin for photo microscopic observations of the liver histopathological structure.

Statistical analysis:

All parameters are expressed as a mean value ± SEM. Differences between the mean value of tests and control groups were evaluated statistically by using the one-way analysis of variance (ANOVA), Student's t-test.

Results and Discussion:

Effect of ethanol extract of *Dioscorea bulbifera* L on serum biochemical parameters:

Effect of EEDB on serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT) and serum alkaline phosphatases (ALP):

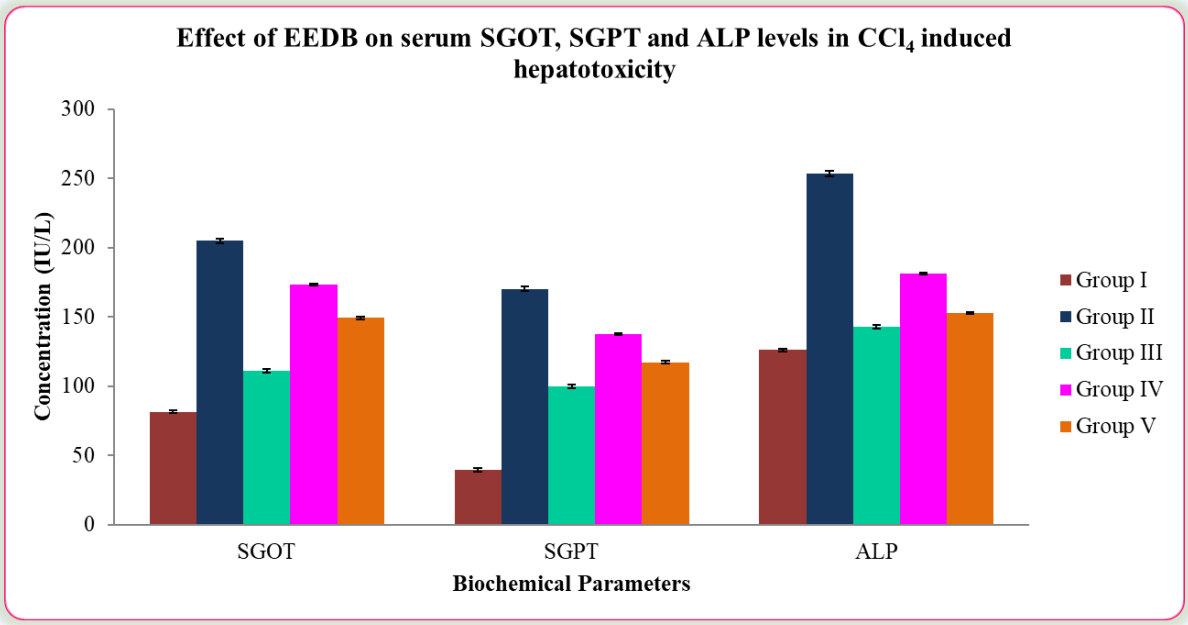

Figure No. 1: Effect of EEDB on serum SGOT, SGPT and ALP levels in CCl_4 induced hepatotoxicity.

Amino transferases are group of liver specific enzymes which are most commonly utilized and considered as very sensitive and specific indicators of hepato cellular necrosis. These enzymes are aspartate amino transferase (AST, formerly serum glutamate oxaloacetic transaminase -

SGOT) and alanine amino transferase (ALT, formerly serum glutamate pyruvate transaminase, SGPT) catalyses the transfer of amino acids of aspartate and alanine respectively to the keto group of glutaric acid [19].

There was a significant increase in the SGOT, SGPT and ALP levels on 28th day in rats treated with CCl_4 (G-II) when compared to the normal group (G-I). The group rats treated with standard drug silymarin (G-III) showed a significant reduction on raised levels of SGOT, SGPT and ALP on 28th day when compared to control (G-II).

The same trend was observed with that of both the groups (IV and V) receiving ethanol extract of *Dioscorea bulbifera* L (200 mg/kg and 400 mg/kg) showed significant decrease in SGOT, SGPT and ALP levels on 28th day when compared to control group. The results shown on Table no. 1 and Figure no 1 suggest that healing of damaged hepatocytes were carried out by plant extract significantly with the high dose of plant extract when compared to low dose of plant extract. The results of the present study indicate that both Test groups probably stabilize the hepatic plasma membrane from CCl_4- induced damage [20 & 21].

Effect of EEDB on serum total bilirubin (TB), total protein (TP) and albumin (ALB):

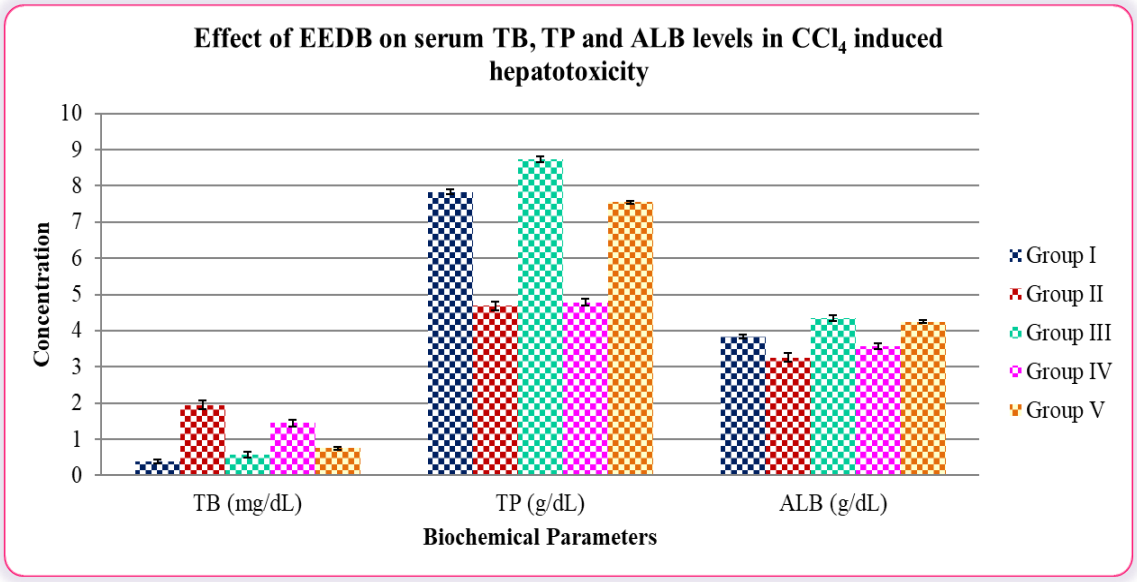

Figure no. 2: Effect of EEDB on serum TB, TP and ALB levels in CCl_4 induced hepatotoxicity

The serum levels of total bilirubin, total protein and albumin were shown on Figure no. 2. A significant increase in the total bilirubin levels and decrease in total protein and albumin levels on 28th day was observed in rats treated with CCl_4 (G-II) when compared to the normal group (G-I). The group (G-III) rats treated with standard drug silymarin showed a significant change i.e., comes to normal levels on 28th day when compared to control (G-II). The trend was same with that of both the groups (IV and V) receiving ethanol extract of *Dioscorea bulbifera* L (200

mg/kg and 400 mg/kg) showed significant decrease in total bilirubin and rise in total protein and albumin levels on 28th day when compared to control group. The results signify that the high dose of plant extract was effective when compared to low dose of plant extract.

Effect of EEDB on serum total cholesterol (TC):

The effect of EEDB on serum levels of total cholesterol were shown on Figure no. 3. A significant rise in total cholesterol levels in CCl_4 treated group (G-II) on 28th day when compared to normal group (G-I). While a significant fall was observed in the total cholesterol levels on 28th day in rats treated with silymarin (G-III) when compared to control group (G-II). With the results the treated groups (IV and V) also witnessed a significant fall in TC levels on 28th day when compared to control group (G-II), indicating the serum cholesterol lowering activity of the extract in a dose dependent manner.

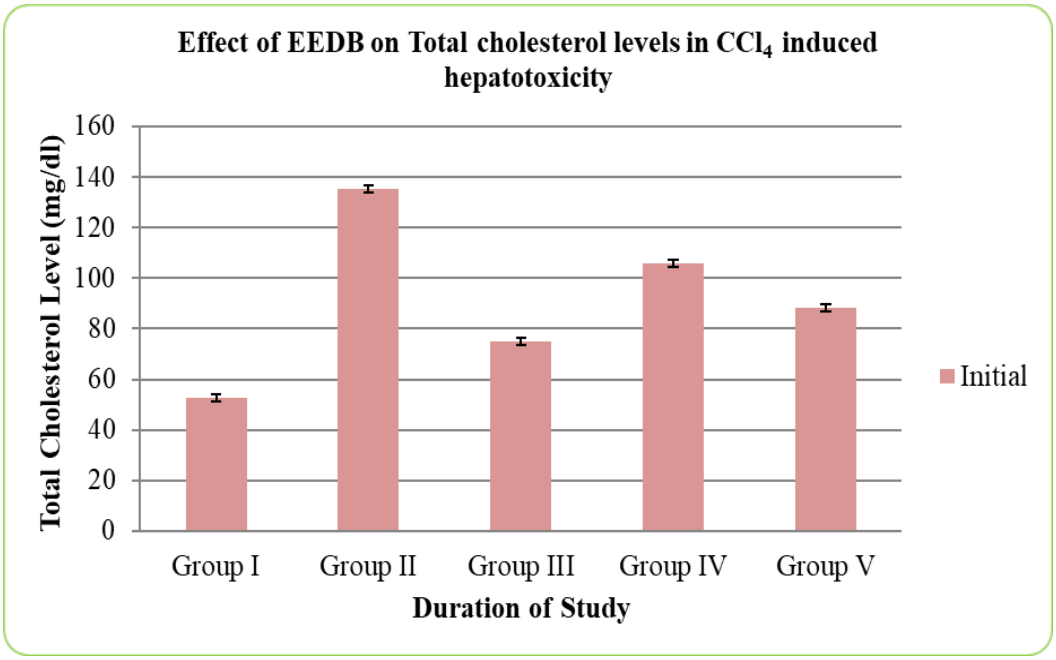

Figure no. 3: Effect of EEDB on Total cholesterol levels in CCl_4 induced hepatotoxicity

In the current study the groups IV and V receiving ethanol extract of *Dioscorea bulbifera* L considerably raised the synthesis of TP by accelerating the regeneration process and protecting the liver cells. The enhanced levels of total protein in serum are indicative of the hepatoprotective activity.

The synthesis of bile acids from cholesterol which is obtained from plasma lipids or synthesized in liver is inhibited during CCl_4 intoxification resulting in increased cholesterol level. The marked decrease in cholesterol levels seen in rats treated with ethanol extracts of *Dioscorea bulbifera* L indicates that the inhibition of bile acid synthesis is reversed.

Effect of EEDB on liver tissue:

Effect of EEDB on SOD, CAT, Red. GSH & MDA in CCl_4 induced hepatic damage

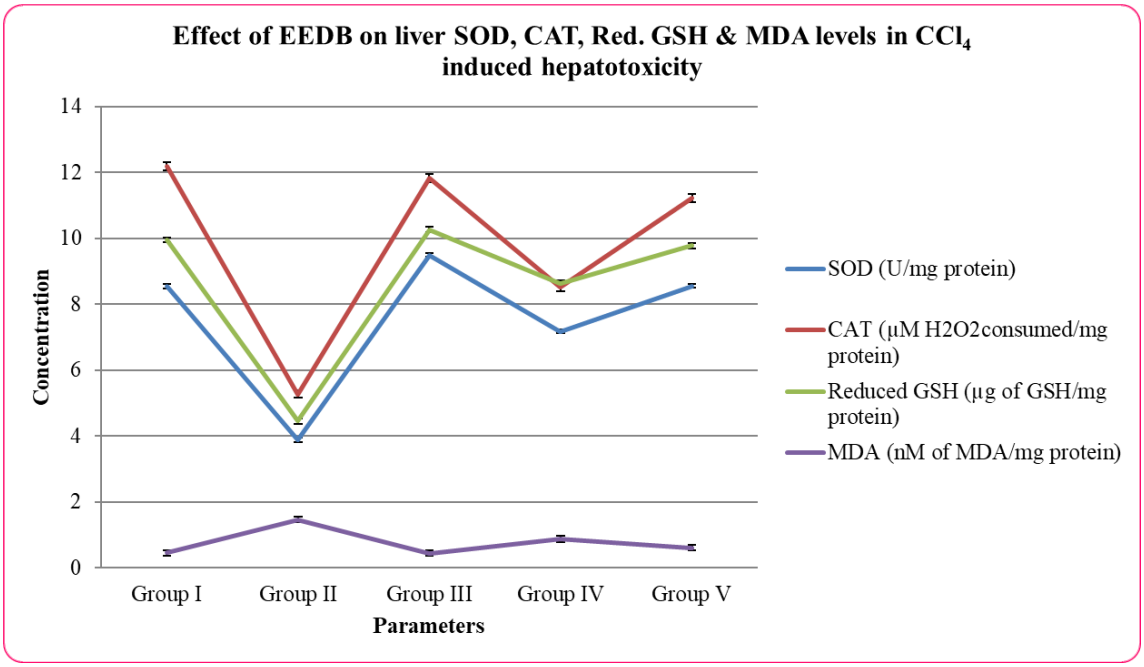

Figure no. 4: Effect of EEDB on liver SOD, CAT, Red. GSH & MDA levels in CCl_4 induced hepatotoxicity

The effect of ethanol extract of *Dioscorea bulbifera* L on SOD, CAT, Red. GSH & MDA levels in CCl_4 induced liver damage were shown on Figure no. 4. There was a drastic and significant decline in the SOD, CAT and reduced GSH levels in rats treated with CCl_4 (G-II) when compared to normal group (G-I). A significant increase in SOD, CAT and reduced GSH levels was seen in rats treated with silymarin (G-III) when compared to control group (G-II). The groups IV and V receiving ethanol extract of *Dioscorea bulbifera* L (200 mg/kg and 400 mg/kg) also showed significant increase in SOD, CAT and reduced GSH levels when compared to control (G-II), implicating its protection against liver damage. There was a significant increase in the malondialdehyde (MDA) levels in the animals of the CCl_4 group (G-II) when compared to the normal group. Whereas a dramatic reduction in the MDA levels was witnessed in case silymarin group. Both the groups (IV and V) have shown a significant reduction when compared to CCl_4 group indicating its ability to prevent the peroxidative degradation of the membrane lipids.

Histopathological studies

Along with the levels of various biochemical parameters the histopathological observations after CCl_4 administration is assessed to find out the extent of hepatic damage caused. When compared to normal hepatic architecture of normal group animals, the control group animals showed severe hepatic damage manifested by intense steatosis, centrilobular necrosis,

ballooning degeneration, nodal formation and fibrosis. Treatment of ethanol extract of *Dioscorea bulbifera* L showed remarkable healing of damaged parenchyma resulting in regenerative effects as shown in Figure 5.

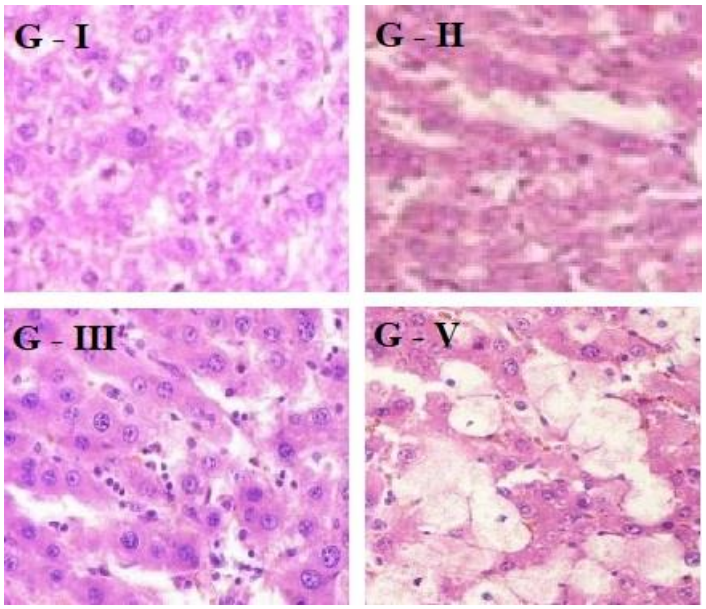

Figure 5: Histopathological observations

Conclusion:

The results exemplified that ethanol extract of *Dioscorea bulbifera* L at both the doses of 200 and 400 mg/kg prevented CCl_4 induced hepatotoxicity in rats. This was evident from the results obtained from the serum biochemical parameters and tissue antioxidant studies. In support to this study, histopathological results also show significant activity of the plant. Finally based on improvement in serum marker enzyme levels, physical parameters, functional parameters and histopathological studies, it is concluded that the ethanol extract of *Dioscorea bulbifera* L possess hepatoprotective activity and thus supports the traditional application of the same under the light of modern science. Present endeavours are coordinated to separate the dynamic constituents which are answerable for its action from different concentrate of plant and explanation of mechanism of action.

ACKNOWLEDGEMENTS

The authors are sincerely thankful to the principal and management of P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, India, for giving vital offices to complete the research work.

FINANCIAL SUPPORT

The authors announce that they have no financing support for this investigation.

CONFLICT OF INTERESTS

The authors proclaim that there was no conflict of interest in this research.

References:

1. Tortora G.J. and Grabowski S.R. (2002) The digestive system (liver and gallbladder) In: Principles of Anatomy and Physiology, seventh edition, Harper Collins college publishers, New york, 24, Page no. 792-795.

2. Navarro VJ, Senior JR. Drug-related hepatotoxicity. The New England Journal of Medicine 354; 731-739; 2006.

3. Friedman, Scott E, Grendell, James H, McQuaid, Kenneth R. Current diagnosis & treatment in gastroenterology, New York: Lang Medical Books/McGraw-Hill. 664-679; 2003.

4. Aashish Pandit, Tarun Sachdeva and Pallavi Bafna. Drug Induced hepatotoxicity: A Review. Journal of applied pharmaceutical sciences; 2(5); 233 – 243; 2012.

5. Ostapowicz G., Fontana R.J., Schiodt F.V., Larson A., Davron J.T., Steven H.B., Timothy M., Reish J. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med. 137: 947–954; 2002.

6. Dominiczak MH. Lipids, lipoproteins In: Baynes JW, Dominiczak MH (eds.) Medical biochemistry, Elsevier Mosby. Philadelphia 234-242; [2005.

7. Ekaidem IS, Akpan HD, Usoh IF, Etim OE, Ebong PE. Effects of Ethanolic Extract of Azadirachta indica Leaves on Lipid Peroxidation and Serum Lipids of Diabetic Wistar Rats. Acta Biological Szegedensis 51; 17-20; 2007.

8. Anand K, Lal UR (2016) Hepatitis and medicinal plants: An Review. 5; 408-415; 2016.

9. Govind pandey. Medicinal plants against liver diseases. International research journal of pharmacy; 2(5); 115 – 121; 2011.

10. U. Subasini1, S. Thenmozhi, D. Sathyamurthy, S. Vetriselvan, G.Victor Rajamanickam and G.P. Dubey. Pharmacognostic and phytochemical investigations of *Dioscorea bulbifera* L. International journal of pharmacy and life sciences. 4(5); 2693 – 2700; 2013.

11. Galani V J, Patel D M. A Comprehensive Phytopharmacological Review of Dioscorea bulbifera Linn. Int J Environ Sci Nat Res. 4(5); 177 – 187; 2017.

12. Lachman and Lieberman, The Theory and Practice of Industrial Pharmacy, 3rd Edn, CBS Publishers and Distributors: 2007, 150-153.

13. N.S.Parmar, Shiv Prakash et.al. Screening methods in pharmacology. Narosa publishing house.2011:279-286.

14. Gopi H Shah et al., Development of carbon tetrachloride- induced chronic hepatotoxicity model in rat and its application in evaluation of hepatoprotective activity of silymarin. Asian journal of pharmaceutical and clinical research. 2017; 10(8).

15. Aebi H, Colowick SP, Kaplane NO (eds). Catalase in vitro In: Meth. Enzymol 1984; 121-126.

16. Moran D, De Buitrago JMG, Fernandez E, Galan AI, Munoz ME and Jimenez R. Inhibition of biliary glutathione secretion by cyclosporine A in the rat: possible mechanisms and role in the cholestasis induced by the drug. J. Hepatol. 1998; 29: 68-77.

17. Slater TF and Sawyer BC. The stimulatory effects of carbon tetrachloride and other halogenoalkanes on peroxidative reactions in rat liver fractions *in vitro*. General features of the systems used Biochemical Journal. 123; 805 – 814; 1971.

18. Misra HP and Fridovichi. The role of superoxide anion in the auto oxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 1972; 247: 3170-3175.

19. Hayes JR, Londie LW, Leeca BJ. Acute 14 days repeated dosage and 90 days subchronic toxicity studies of CCl_4 in CDI mice. Fundam Appl Toxicol; 7; 4-54; 1986.

20. Thabrew M and Joice P. A comparative study of the efficacy of *Pavetta indica* and *Osbeckia octanda* in the treatment of liver dysfunction. Planta Med. 1987; 53: 239-241.

21. Burtes CA, Ashwood ER. Textbook of clinical chemistry. Philadelphia: WB Sunders Co; 1986: p. 56.