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Hepatoprotective Effect of Lipoic Acid in Ethanol Intoxicated Chicks on Liver Biochemical Parameters Like LDH, SDH, & Glucose-6- Phosphatase

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Abstract

Aim of the study is to investigate the effect of ethanol by the action of lipoic acid on certain liver parameters like lactate dehydrogenase, succinate dehydrogenase, and glucose-6-phosphatase etc. Scientific outcome that lipoic acid possess hepatoprotective as well as antioxidant properties. During the study we found that all parameters are up to the mark and scientific outcomes ensures the quality of the work. Hence this study needs attention further for its practice in real pharma as well as scientific industries to deliver a quality outcome for a society.

Keywords: Hepatoprotection, Lipoic Acid, Cheeks, Ethanol.

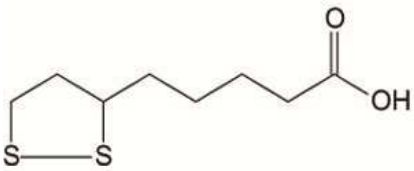
INTRODUCTION

For the benefit of recipient drug is used to modify, cure or explore physiological or pathological states[1]. These are the natural or synthetic substances not only provide the structural basis and energy to living organism, but regulate their functional activities. The interaction between potent chemicals and immune system contribute in understanding the life processes and provide the effective method for treatment, prevention and diagnosis of many diseases.[2]Alpha lipoic acid (ALA) is a fat soluble, sulfer containing, vitamin- like universal antioxidant. Because it has both properties hepatoprotective as well as antioxidant. It is not a true vitamin because it can be synthesized in the body and is not necessary in the diet of animal. Alpha Lipoic acid is found as an important growth factor for many bacteria and protozoa it is the most active form of lipoic acid.[3] Alpha lipoic acid is a disulphide derivative of octonoic acid found naturally in mitochondria as the co-enzyme for pyruvate dehydrogenase (PDH) and alpha-ketoglutarate dehydrogenase (α-KGDH). Alpha lipoic acid can be characterized as an efficient and unique antioxidant. Thus, it can confer free radical protection to both interior and exterior cellular structures.

The unique physico-chemical properties of a-lipoic acid make it a powerful and reactive biological molecule, chosen by evolution to carry out biochemical reactions crucial for oxidative metabolism, and shown researchers to modulate various cellular functions.[4]

The uses of lipoic acid in the treatment of diabetes, suppressed HIV replication and inhibition of HIV by reducing the activity of reverse transcriptase it should be used for the treatment for stroke, it slow down the ageing process, give protection against heart diseases and cancer, improves skin and health, erase wrinkles, regulate blood sugar in diabetes.[5]Lipoic acid a sulfur containing compound of vitamin like enzyme cofactor, is an essential nutrient in metabolism. It plays the crucial role in energy reactions that turn both fat and carbohydrate into energy. Lipoic acid is a metabolic antioxidant fat soluble. It also enhance and recycle other antioxidants. It is used for detoxification of heavy metal poisoning in the body. The general formula of lipoid acid is $C_8H_{14}O_2S_2$ and having a molecular weight 206.32 it is also known as Thioctic acid or 6, 8-Dithiooctanoic acid and melting and boiling point of lipoic acid respectively M.P.

60-62°C and B.P. 160-165°C the structural formula of lipoic acid is as follows.



Lipoic acid

Excess of any compound will be harmful to life and considered under toxicity studies. Toxicology is th study of symptoms, mechanism, treatments and detection of poisoning[6] chronic alcoholism produces a wide spectrum of liver and other organ diseases depending on the amount of duration of alcohol intake. The liver affection ranges from fatty changes to hepatitis and cirrhosis. Protein deficiency and enzyme activity depression were proved to be associated with alcoholic liver diseases.It should be characterized by a changes in concentration of the enzyme activities. ALA should be used as a therapeutic agent in a number of conditions relating to liver disease, including alcohol induced damage, metal intoxification, and CCl_4 poisoning α-lipoic acid supplementation was successful in the treatment for these conditions in many cases.[7]

Present study was undertaken to find out hepato protective effect of lipoid acid in avian model. The compound was given orally to the broiler chicks, with the help of canula and determined LD-50. LD50 (Lethal dose) is the dose that kills half (50%) population of animal tested. It is one way to measure the short term poisoning potential (acute toxicity) of a material.[1]

The protective effects of α-lipoic acid against free radical-mediated injury interested several groups in examining weather lipoic acid or dihydrolipoic acid protects hematopoietic tissues. Free radical damage induced by ionizing radiation. Recently, protective effects of α-lipoic acid against radiation damage were observed by Ramakrishnan *et al*., (2001)[8]

Liver is a vital organ present in animal's body and primary site for metabolism of any toxic substances and suffered first. The liver plays very important role in avian metabolism and other animals. So that any change in liver system will definitely affect complete metabolism of an animal.[6] Although its structure is simple, but it performs most complex functions.[9] It performs various roles such as maintenance, regulation of homeostasis of the body. It acts with almost all the biochemical pathways to growth, energy, provision, reproduction, fight against diseases, nutrient supply.[10] It has wide range of function including detoxification, protein synthesis and production of biochemical's necessary for digestion, storage of vitamins, carbohydrate metabolism for overall health and well being, to maintain the healthy liver is a critical factor as it continuously exposed to environmental toxins and alcohol and lead to various diseases.[11]

In this study we have studied effect of Lipoic acid treatment on Liver enzymes of alcohol intoxication. Lactate dehydrogenase (LDH) is a ubiquitously intracellular enzyme which catalyzes the reversible oxidation of lactate to pyruvate with the nicotinamide adenine dinucleotide (NAD) serving as co-enzyme.[12]

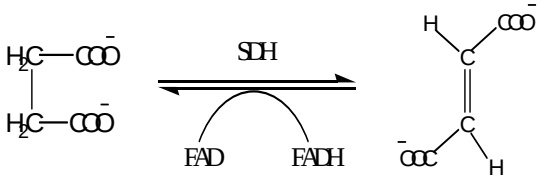
Lactate + NAD ----------> LDH Pyruvate + NADH

Generally liver, skeletal muscle, heart shows high concentration of LDH.[13] The specified factor leading to LDH inhibition in chronic alcoholism were the increased "NADH/NAD" ratio causing shift to the left in the equilibrium of the oxidoreductive couple lactate-pyruvate resulting in hyperlactacidaemia.[14] The extracellular release of LDH could lead to liver LDH lowering.[15]

Most of mitochondrial LDH was solubilised by sonication of the mitochondrial fraction in 0.15 M NaCl, pH 6. The total extracted LDH activity was 3-fold higher than the initial pellet activity.[16] LDH is essential for anaerobic glycolysis and hence necessary for muscular work in the absence of oxygen.[17] In chickens with clinical leucosis increased concentrations of LDH were reported[18].LDH was chosen for this study because a good deal of information is available about its enzymatic characteristic and genetic control.

Another enzyme under study is succinate dehydrogenase. Succinate dehydragenase is the enzyme of TCA cycle. It catalyses the conversion of succinate to fumarate, in this reaction 2-hydrogen atoms are removed from

succinate by co-enzyme FAD which is ultimately reduced to 1, 3, 5 triphenyl pharmazones. It is a coloured compound (i.e. pink) and can be colourimetrically estimated very easily.



Succinate Fumarate

The activity of SDH an oxidative enzyme involve din the Krebs cycle, was significantly decreased in muscle and kidney tissues after corroborating earlier findings with sodium fluoride exposure. SDH activity of all tissues in the present study clearly indicates depletion in the oxidative metabolism at the level of mitochondria leading to depression of TCA cycle under ethanol and Lipoic acid exposure.

Similarly, glucose-6-phosphotase is another enzyme under study. It acts on glucose-6-phosphate and convert it into glucose and inorganic phosphate. This inorganic phosphate librated is estimated by Fiske and Subbarao's method.

Glu-6-Ptase
Glucose-6-phosphate ----------> Glucose + Inorganic phosphate

G6PD is remarkable for its genetic diversity. Many variants of G6PD, mostly produced from missense mutations, have been described with wide ranging levels of enzyme activity and associated clinical symptoms. G6PD deficiency is very common worldwide, and causes acute hemolytic anemia in the presence of simple infection, ingestion of fava beans, or reaction with certain medicines, antibiotics, antipyretics, and antimalarials.

MATERIALS AND METHODS

The study was carried out at the department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola.

Experimental Mehtod:

A total of 103, one day old broiler chicks were purchased from Sidhant Poultry, Akola (Maharashtra). The study was started after acclimatization when birds gained weight of 130-150 g. They were housed in well ventilated room in hygienic condition during the period of experiment. The birds were then divided into 5 groups. Each group consist of 5 bird and marked them as A, B, C, D and E. The body weight was recorded daily before drug treatment and also before scarification. The ALA doses were given to checks as per the body weight of check orally for 21 days and after scarification the blood sample and organ (liver) were collected in ice cold conditions for biochemical and enzymatic assay.

LD_{50} Determination:

In toxicology, the median lethal dose, of a toxic substance or radiation is dost required to kill half the number of tested population after a specified test duration.[19] Sets containing, 4 birds each were taken and doses were given orally through cannel. The birds were observed for 96 hours and then mortality was noted for further calculation, from this we calculated exact value of LD_{50} of each compound. The 50% of LD_{50} was considered as effective dose in chicks. The LD_{50} and effective dose of each compound is shown in the following table.

Table 1. LD_{50} for Ethanol intoxification

Sr.No.	Drug	LD_{50}%	Effective Dose
1	Ethanol	7 ml / kg. 10 ml / kg. 12 ml / kg.	5 ml / kg.

Table 2. LD_{50} for Lipoic Acid Treatment

Sr.No.	Drug	LD_{50}%	Effective Dose
1	Lipoic acid	150 mg / kg. 200 mg / kg. 300 mg / kg.	100 mg / kg.

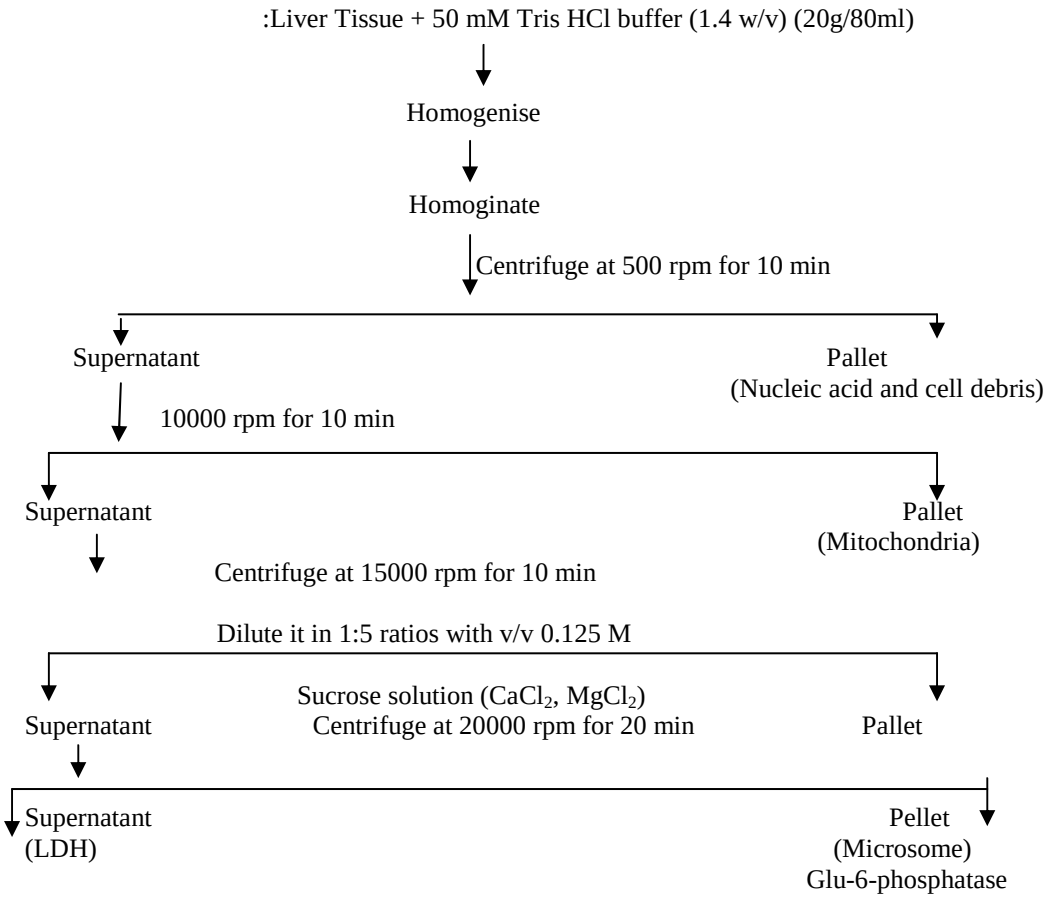
Preparation of Liver Homogenate:

The chicks were sacrificed by cervical dislocation and their liver were per fused with 0.9% ice cold saline and removed immediately and placed in saline. Homogenized in 50 mM tris HCl buffer (1:4 w/v) pH 7.4 containing 1.15% KCl, using a motor driven Teflon pestle glass homogenizer. Before it the liver were minced with sharp pair of scissors, the blood was washed off and pieces of liver homogenized in suitable volume of isolation medium for about 3 min using an electrically operated lab type homogenizer.

Estimation of Liver Enzyme:

The liver homogenate was placed in centrifuge tubes and spun in a refrigerated centrifuge at 3000 rpm for 10 min. The supernatant was carefully collected and centrifuge at 8000 rpm for 10 min. The resultant supernatant was collected. From the mitochondrial pallet succinate Dehydrogenase was assayed. The collected post mitochondrial fraction centrifuge at 11000 rpm for 10 min the lysosomal fraction was obtained. The resultant supernatant suspended in 20 ml of suspension medium consist of 0.0125 M sucrose and 8.5 M $CaCl_2$ and 5.5 mM $MgCl_2$ and centrifuge at 20000 rpm for 10 min.

The final supernatant was suspended in suspension medium to a final volume of 5 ml. The microsomal fraction contains glucose-6-phosphate in pallet and in supernatant lactate Dehydrogenase was obtained. All isolation procedure was performed at 0-4°C.The general representation of the summary of SDH, LDH and glucose-6-phosphatase isolation is as follows



Enzyme Assay:

The activity of LDH was measured by Robonic make pretest touch Biochemical semi Automated Analyzer by decrease in optical density at 340 nm according to the method of (L→P) UV-kinetic manufactured by Enzopak Company. In any assay the rate of reaction can be determined by measurement of amount of product or substrate utilized. As the amount of product formed in unit time of LDH, the substrate is Lactate & the product is pyruvate.

Table.3 Preservations

Preservation	**No. of Bottles/Pouches**
Store all reagents at 2-8°C	20 x 1.1 ml
* 1 LDH (Coenzyme)	2 (10 tablet)
* 2 LDH (Buffered Substrate)	2
Reconstitution vial	1

Precaution:

ENZOPAK LDH (L → P) is for In Vitro diagnostic use only.

Working Reagent Preparation:

Reconstitute one vial of 1 LDH with 1.1 ml of 2 LDH. Mix gently to dissolve the contents. Use after 5 min.

Reagent Storage and Stability:

Enzopak LDH (L → P) reagents are stable at 2-8° C until the expiry date stated on the label. Reconstituted reagent is stable at 2-8° C for 2 days.

Specimen Collection:

Fresh, clear, serum with no hemolysis is essential.

Table 4. Reaction Parameters

Reaction Parameters:	
Type of Reaction	Kinetic/Increasing OD
Wavelength	340 nm
Flow cell Temperature	37° C
Delay Time	60 Seconds
Interval Time	30 Seconds
Number of Readings	4
Reagent Volume	1.0 ml
Sample Volume	50 Microlitres (0.05 ml)
Factor	3376
Zero setting with water	Distilled
Path length	1.0 cm

Table 5.Protocol 1

Sr.	**Pipette into test tubes**	**Test**
1	Working reagent (ml)	1.0
2	Sample (ml)	0.05

Mix and read first absorbance of the test exactly at one min and thereafter at 30, 60 and 90 seconds at 340 nm. Determine the mean change in absorbance per min and calculate test results.

Estimation of Liver SDH

Enzyme Assay:

The SDH activity was measured by UV-VIS spectrophotometer as increased in optical density at 440 nm. The rate of reaction can be observed by measuring amount of product produced or substrates utilized, but in case of SDH the substrate succinate is converted to fumarate by lose of two hydrogen. It is an enzyme of TCA cycle.

Table 6.Protocol 2

Sr.	Reagents	Blank	Std.	Exp.	Const
1	Phosphate Buffer (ml)	--	--	0.5	0.5
2	Sodium Succinate (ml)	--	--	0.5	--
3	Liver Homogenate (ml)	--	--	0.5	0.5
4	Distilled Water (ml)	3.00	0.5	0.5	1
5	TTZ (0.1%) (ml)	--	--	1	1
6	TTZ (100 ug/ml) (ml)	--	0.5	--	--
	Add few crystals of sodium dithionate to Std. and Blank and mix well keep at 37°C for 30 minutes.				
7	Acetone (ml)	7 ml	7 ml	7 ml	7 ml
	Tubes were stop and shaken vigorously centrifuge take supernatant, take optical density at 440 nm				
8	Optical Density at 440 nm	0.00	1.0	S- 0.36 P-0.68	S-0.20 P-0.56

Assay of Glucose-6-Phosphatase:

The activity of glucose-6-phosphatase was measured by Fisk and Sobaros method and using UV-VIS Spectrophotometer at 660 nm. The glucose-6-phosphatase acts on glucose-6-phosphate and convert it into glucose and inorganic phosphate.

Glu-6-Phosphatase.
Glucose-6-PO_4 ----------→ Glucose + Inorganic phosphate

Table 7.Protocol 3

Sr.	Reagents	Control (ml)	Exp. (ml)
1	Tissue suspension	0.1	0.1
2	Glu-6-PO4	--	0.1
3	Citrate Buffer	0.2	0.2
	Incubated at 37°C for 15 min		
4	TCA 10% (ml)	2	2

Table. 8 Protocol 4

Sr.	Reagents	Blank	Std.	Const.	Test
1	Supernatant	--	--	1	1
2	Std. Phosphorus	--	0.2	--	--
3	Distilled water	1	0.8	--	--
4	Ammonium Molybdate	5	5	5	5
5	ANSA	1	1	1	1
	Keep at room temperature for 15 min				
6	Optical Density at 660 nm	0.00	0.05	0.50	0.54

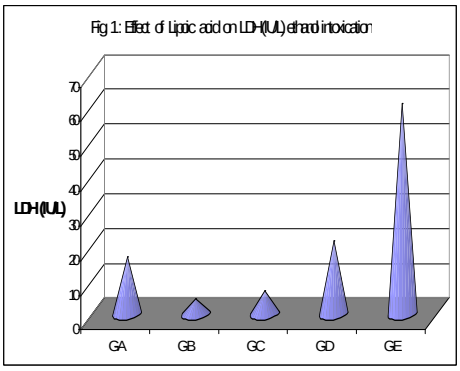
RESULT

The liver enzyme LDH, SDH and glucose-6-phosphatase results are shown in following table no 7

Table 9. Results

Sr. No.	Enzyme	G-A	G-B	G-C	G-D	G-E	Avg %	Std. Deviation	Relative Std Deviation
1	LDH (IU/L)	16.37	4.55	6.58	21.1	60.76	21.87	22.78	1.04
2	SDH (mM/L)	2.26	2.00	2.5	1.33	2.06	2.03	0.43	0.215
3	Glu-6-Phosphatase (mM/L)	9.14×10^{-4}	11.29×10^{-4}	15.40×10^{-4}	16.52×10^{-4}	20.10×10^{-4}	14.49	4.33	0.29

Group – A	**---**	***Control***
Group – B	**---**	**Alcohol**
Group – C	**---**	**Lipoic acid**
Group – D	**---**	**Alcohol + Lipoic a (3 weeks) (3 weeks)**
Group – E	**---**	**Alcohol + Lipoic acid (2 weeks) (1 week)**



The ethanol ingestion shows significant decrease in LDH level as indicated in figure 1. While the level of enzyme shows gradual increase in group C to group D. While a tremendous rise in activity of LDH was observed in group E (Ethanol 2 week + Lipoic acid for 1 week)

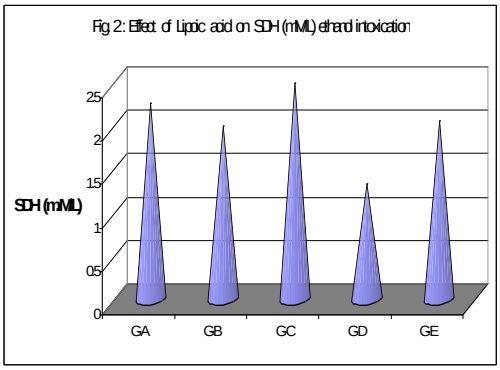
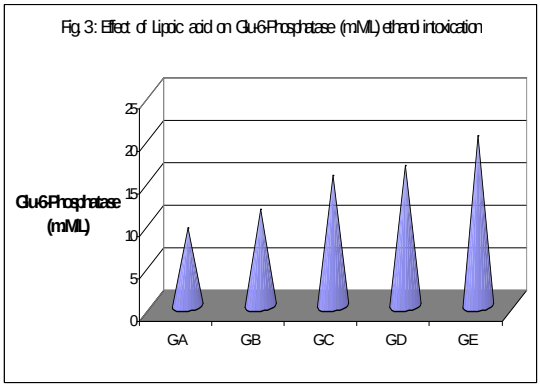


Figure 2 shows the effect of ethanol and lipoid acid on SDH levels. A slight decreased was observed in group B i.e. on treatment with plain ethanol. While group C shows slight increased in activity as compared to control. However a significant decrease in group D was observed (Alcohol + lipoic acid for 3 weeks simultaneously). Similarly marginal decrease was noted in group E (Alcohol for 2 weeks + lipoic acid for 1 week).



In figure 3 indicates the levels of glucose-6-phosphatase in various groups. It shows a gradual rise in activity of enzyme from group A, B, C, D and E respectively.

DISCUSSION

Hepatoprotective study on ethanol intoxicated broiler chicks caused significant changes, in liver tissue enzyme, and in blood which is treated with universal antioxidant known as α-lipoic acid. Liver is a primary site of detoxification and is generally major site of intense metabolism. It is also site of biotransformation by which toxic compounds has been transformed in less harmful form to reduce toxic.[20] The present chronic ethanol toxicity study recorded significant decrease in hepatic SDH, LDH, and G-6-Phosphatase activity. These decreased activities were in accordance with previous researches. These lowered enzyme activities could principally be explained by the toxic actions of ethanol on proteins and on hepatocytes cellular organelles in general. Ethanol itself forms a toxic environment favorable to oxidative stress such as hypoxia, endotoxeamia and cytokines release. Ethanol also interpolating the biomembranes and expanding them increases membrane fluidity and enzyme release[21]. Ethanol is metabolized to acetate by alcohol dehydrogenase and aldehyde dehydrogenase, using NAD Acetaldehyde and the increased production of NADH and hydrogen ions are believed to be responsible for many of the hepatotoxic effects of ethanol the beneficial effect of α-lipoic acid can be explained by the opposing actions of alcohol and α-lipoicacid on the NADH/NAD ratio: alcohol lowering this ratio, in contrast to a-lipoic acid, which increases this ratio via its cellular reduction to dihydrolipoic acid using NADH and NADPH to produce NAD and NAPD.[22]

The specified factors leading to LDH inhibition in chronic alcoholism were the increased "NADH/NAD" ratio causing shift to the left in the equilibrium of the oxidoreductive couple lactate-pyruvate resulting in hyperlactacidaemia. The extracellular release of LDH could lead to liver LDH lowering. This found support the statement that chronic alcoholism caused extracellular release of hepatic LDH raising its activity in plasma in present study suggest the activity of LDH is increased in group: A,D, and E but in case of group: B and C the level of LDH is decreased.

The extracellular release of LDH could lead to liver LDH lowering that chronic alcoholism caused extracellular release of hepatic LDH raising its activity in the plasma. Thus the decreased SDH activity was disturbed by structural and functional integrity of hepatic orgenells. The depression of citric acid cycle fairly contributes to SDH lowered activity. The increased activity of glucose-6-phosphatas enzyme due to acetaldehyde is not librated and glucose-6-phosphatase enzyme transport protein (translocose, T_1) is not inhibited. The elevated cytosolic calcium microsomal fraction.

Hence it is absolutely correct the lipoic acid work as a antioxidant and hepatoprotective.

CONCLUSION

Lipoic acid treatment successfully ameliorated the toxic effect of ethanol on liver enzyme LDH, SDH and Glucose-6-phosphatase by variable degrees according to the ways and the period of administration.

The studied parameters help to learn more about the changes that took place in liver and to identify the potential problems of alcohol.

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