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Evaluation of polyherbal formulation, livomyn for it's hepatoprotective and antioxidant activity

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ABSTRACT

A Polyherbal formulation, Livomyn comprising of phytoconstituents with potential hepatoprotective activity was evaluated for its hepatoprotective and antioxidant activity using Carbontetrachloride (CCl_4) induced hepatotoxicity in Sprague Dawley rats. Livomyn is composed of the extracts of plants like Andrographis paniculata, Phyllanthus niruri,Triphala, Boerhaavia diffusa, Amoora rohituka, Chicorium intybus, Adhatoda vasica, Eclipta alba, Zingiber officinale,Berberisaristata,Fumariaofficinalis,Embelliaribes,Tephrosiapurpurea,TinosporacodifoliaCoriandrumsativum,Aloebarbadensis,Picrorrhizakurroa.Hepatotoxicity was induced in Sprague Dawley rats by intraperitoneal injection of CCl_4(1.5mL kg^{-1},60 in olive oil,1:1 ratio). Livomyn at a dose of 120, 240, 480 mg/kg/day and Silymarin standard 50mg/kg/day was administrated orally for 7 days.The Hepatoprotective effect of Livomyn and standard was evaluated by the assay of biochemical parameters viz…Serum Glutamate Pyruvate Transaminase (SGOT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline phosphate (ALP), Total Bilirubin Protein (TBP), whereas DPPH Scavenged % was estimated to evaluate antioxidant activity. The toxic effects of CCl_4 in Livomyn treated group was controlled significantly by restoration of the levels of serum bilirubin protein, enzymes as compared to the CCl_4 treated and silymarin treated groups. Livomyn showed significant hepatoprotective activity as indicated by a decrease in serum marker enzymes (SGOT, SGPT and ALP and increase TBP in a dose dependant manner. Histopathological studies further confirmed the hepatoprotective activity of Livomyn. The present findings are indicative of the hepatoprotective effects of Livomyn against CCl_4 induced oxidative damage being related to its antioxidant and free radical scavenging activity.

Key words: Livomyn, Antioxidant, CCl_4, DPPH Scavenged %, Biochemical parameters.

INTRODUCTION

Liver plays a major role in detoxification and excretion of many endogenous and exogenous Compounds. Any impairment to its function may lead to many implications on one's health. Management of liver disease is still a challenge to the scientific community [1].Conventional or synthetic drugs used in the treatment of liver diseases are often inadequate and can have serious adverse effects. As a result, there is a worldwide trend to go back to traditional medicinal plants. Many natural products of herbal origin are in use for the treatment of liver ailments [2]. Carbon tetrachloride (CCl_4) is a potent hepatotoxin producing centrilobular hepatic necrosis, Liver cirrhosis, tumors, fatty liver and also kidney damage on chronic exposure [3]. The polyherbal formulation, Livomyn is composed of extracts of plants like *Andrographis paniculata, Phyllanthus niruri,Triphala, Boerhaavia diffusa , Amoora rohituka, Chicorium intybus, Adhatoda vasica,* Eclipta alba, *Zingiber officinale,Berberis aristata, Fumariaofficinalis, Embellia ribes,Tephrosia purpurea,Tinosporacodifolia, Coriandrumsativum, Aloebarbadensis,Picrorrhiza kurroa.* Plants rich in polyphenolic compounds are known to be excellent antioxidants *in vitro* and have the capacity to scavenge free radicals and potentiate antioxidant defenses. Studies have been carried out on all the above herbs with antioxidant approach to manage various diseases [4].

The aim of the present study was to investigate the effects of Livomyn on Liver function in CCl_4 induced hepatotoxicity and to make an attempt to understand the probable mechanism involved in producing hepatoprotective effects.

MATERIALS AND METHODS

Experimental Animals:
The animals used for experimentation were Sprague Dawley rats weighing between 170-200gm obtained from the albino enterprises, Hyd. They were housed in clean polypropylene cages under standard conditions of temperature (25 ± 20^0C) and light (12 h light/12 h dark cycle) and fed with a standard diet (Baramati agro pvt.ltd, pune, India) and water *ad libitum.* All animals were handled with human care. Experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (Animal House Registration No. 25/50/CPCSEA).

Drugs and Chemicals:
Silymarin was purchased from Microlabs. DPPH and Gallic acid were purchased from Sigma Aldrich Company (U.S.A). Livomyn from Charak Company, CCl_4 from Star chemicals (A.P), and Olive oil from SDFCL Company and ethanol from Changshu yangyuan chemicals (China) were purchased. A standard kit (Autospan) was obtained from Span diagnostics Ltd (Gujarat).

Preparation of Test and Reference drug solutions:
Polyherbal formulation, Livomyn and Silymarin were suspended in an aqueous solution of 1% carboxymethylcellulose (cmc) daily prior to administration.

Experimental:
CCl_4 induced hepatotoxicity:-
Sprague-Dawley rats weighing in the range of 170-200gm were divided into five groups of six animals each.
Group1: Normal control group- received distilled water for 7 days.
Group2: Toxin control group-received distilled water for 6 days and on 7^{th} day received single dose of CCl_4(1.5mL kg^{-1},60 in olive oil,1:1 ratio), intraperitonially.
Group3 and Group4: Both the groups were treated daily with Livomyn orally for 7 days and on the 7^{th} day received a single dose of CCl_4(1.5mLkg^{-1},in olive oil,1:1 ,30 min after the administration of Livomyn.
Group5: Standard group-treated with silymarin(50mg/kg/day) daily for 7 days and on the 7^{th} day received a single dose of CCl_4 (1.5Ml,in olive oil,1:1, 30min after the administration of silymarin.

At the end of the experimental period, rats were deprived of food overnight and sacrificed by decapitation. Blood samples were collected and allowed to clot for 30-40min. Serum was separated by centrifugation at 37^0c and was used to estimate various bio-chemical parameters [5].

Estimation of Marker Enzyme Assay:
The lysosomal enzymes SGOT, SGPT, ALP, Total Bilirubin protein (TBP), were assayed in Serum using standard kits supplied by Span Diagnostics. The results were expressed as IU/l for SGOT, SGPT and ALP, whereas g/dl for TBP.

Estimation of Antioxidant activity by DPPH Radical scavenging method:
The free radical scavenging activity of Livomyn was measured in vitro by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay. About 0.1 mM solution of DPPH in 100% ethanol was prepared and 1 ml of this solution was added to 3 ml of Livomyn dissolved in ethanol at different concentrations (10–100 µg/ml). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517nm using a spectrophotometer.

The IC_{50} value of the drug was compared with that of ascorbic acid, which was used as the standardThe capability to scavenge the DPPH radicals was calculated using the following formula,

$$\text{DPPH scavenged (\%)} = \frac{(\text{A cont} - \text{A test}) \times 100}{\text{A cont}}$$

Where,
A cont is the absorbance of the control reaction mixture.
A test is the absorbance of sample at different concentrations [6].

Histopathological Studies:
Animals were sacrificed on the day of withdrawal of blood and the livers were aseptically removed, sliced and washed with ice cold saline. Liver sections were fixed in 10% formalin solution. After dehydration, the pieces of liver were embedded in paraffin wax, cut into 4–6 μ m thick sections and stained using haematoxylin and eosin. They were observed under a microscope for histopathological changes in liver architecture and photographed.

Statistical Analysis:
The results of hepatoprotective and antioxidant activities are expressed as mean ± SEM. The statistical analysis of the results were carried out with Graph Pad INSTAT version 3 software and based on Analysis of Variance (ANOVA), one way ANOVA followed by Dunnet's multiple comparisons test. Statistical significance was set at $p < 0.05$.

RESULTS

Following CCl_4 induced hepatotoxicity; a marked increase was observed in the serum SGOT, SGPT and ALP levels with a significant decrease in TP levels, treatment with Livomyn showed a significant reduction in serum SGOT, SGPT and ALP levels and an increase in TP level in dose dependant manner as shown in **table 1**, **table 2, and table 3.**

Table 1: Effect of Livomyn on biochemical serum parameters in Carbon tetrachloride induced Hepatotoxicity on 0th day

Treatment groups and dose (mg/kg)	SGOT (IU/l)	SGPT (IU/l)	ALP (IU/l)	TB(g/dl)
Normal control	51.7±0.45	44.9±0.65	138.7±0.52	0.76±0.03
Disease control	82.8±0.44 a**	85.6±0.64 a**	186.9±0.58 a**	1.57±0.34 a**
Standard	57.6±0.45 b**	55.0±0.75 b**	147.5±0.46 b**	0.84±0.03 b**
Low dose(120)	73.5±0.42 b*	73.8±0.39 b**	179.8±0.70 b*	0.96±0.034 b**
Medium dose(240)	71.2±0.42 b**	70.0±0.56 b**	167.1±0.76 b**	0.96±0.035 b**
High dose(480)	61.8±0.50 b**	59.7±0.87 b**	159.1±0.84 b**	0.89±0.006 b**

*N = 6, each data suggest Mean ±SEM; One-way ANOVA followed by Dun net's multiple comparison test is applied for statistical analysis; a = toxicant groups was compared with control group, b = treated groups were compared with toxicant group; *Significant at $p < 0.05$, ** Significant at $p < 0.01$.*

Table 2: Effect of Livomyn on biochemical serum parameters in Carbon tetrachloride induced Hepatotoxicity on 4th day

Treatment groups and dose (mg/kg)	SGOT (IU/l)	SGPT (IU/l)	ALP (IU/l)	TB(g/dl)
Normal control	51.9±0.52	45.8±0.42	139.5±0.56	0.75±0.04
Disease control	78.6±0.54 a**	83.6±0.80 a**	188.3±0.60 a**	1.13±0.18 a**
Standard	68.3±0.42 b**	64.8±0.64 b**	153.0±0.52 b**	0.96±0.03 b**
Low dose(120)	84.6±0.53 b**	83.9±0.46 b*	192.9±0.62 b**	0.97±0.02
Medium dose(240)	82.1±0.63 b**	82.9±0.76 b**	182.9±0.45 b**	0.97±0.03 b**
High dose(480)	72.8±0.73 b**	73.7±0.54 b**	163.6±0.39 b**	0.96±0.004 b**

*N = 6, each data suggest Mean ±SEM; One-way ANOVA followed by Dunnet's test is applied for statistical analysis; a = toxicant groups was compared with control group, b = treated groups were compared with toxicant group; * Significant at $p < 0.05$, ** Significant at $p < 0.01$*

Table 3: Effect of Livomyn on biochemical serum parameters in Carbon tetrachloride induced Hepatotoxicity on 7th day

Treatment groups and dose (mg/kg)	SGOT (IU/l)	SGPT (IU/l)	ALP (IU/l)	TB(g/dl)
Control	51.76±0.39	41.73±0.50	139.1±0.45	0.72±0.026
Toxicant	75.63±0.49 a**	75±0.62 a**	186±0.39 a**	0.94±0.034 a**
Standard	58.63±0.43 b**	60.4±0.27 b**	149±0.39 b**	0.84±0.003 b**
Low dose(120)	78.48±0.42 b*	76.5±0.61 b**	181.5±0.45 b*	0.94±0.03
Medium dose(240)	72.71±0.48 b**	73.5±0.58 b**	168.5±0.45 b**	0.97±0.066 b**
High dose(480)	66.12±0.47 b**	62.6±0.50 b**	157.1±0.53 b**	0.88±0.042 b**

*N = 6, each data suggest Mean ±SEM; One-way ANOVA followed by Dunnet's test is applied for statistical analysis; a = toxicant groups was compared with control group, b = treated groups were compared with toxicant group; * Significant at $p < 0.05$, ** Significant at $p < 0.01$*

Fig 1: Graph of SGOT values on 0th, 4th, 7th day:

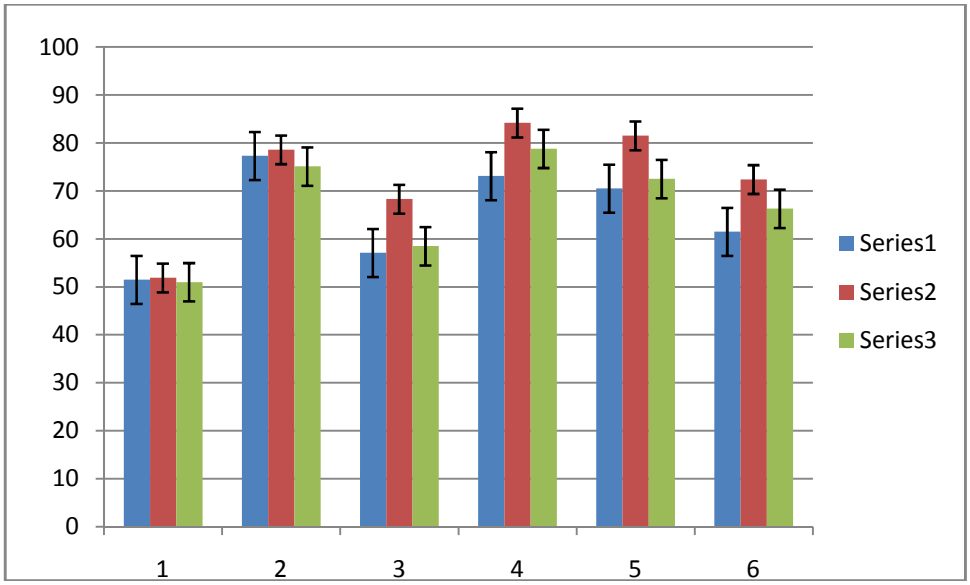


Fig 2: Graph of SGPT values on 0th, 4th, 7th day:

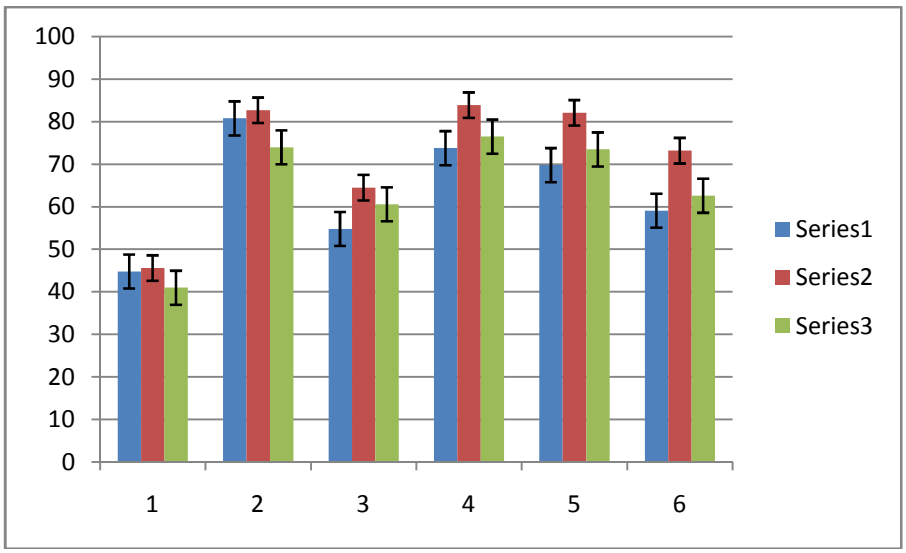


Fig 3: Graph of TOTAL BILIRUBIN values on 0th, 4th, 7th day :

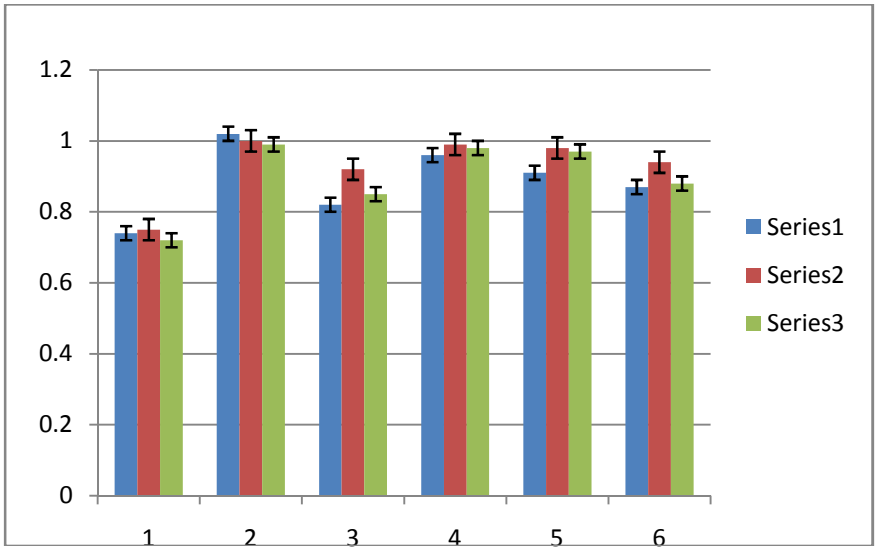
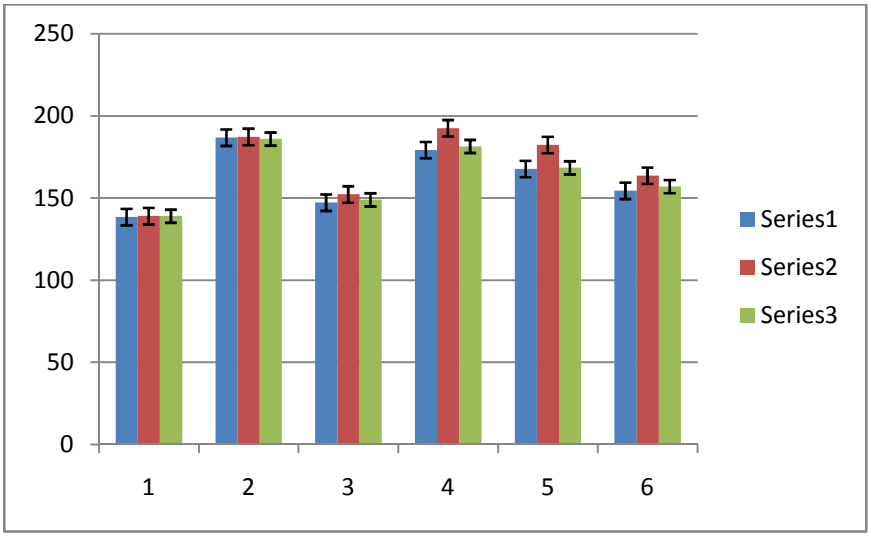


Fig 4: Graph of ALP values on 0th, 4th, 7th day:



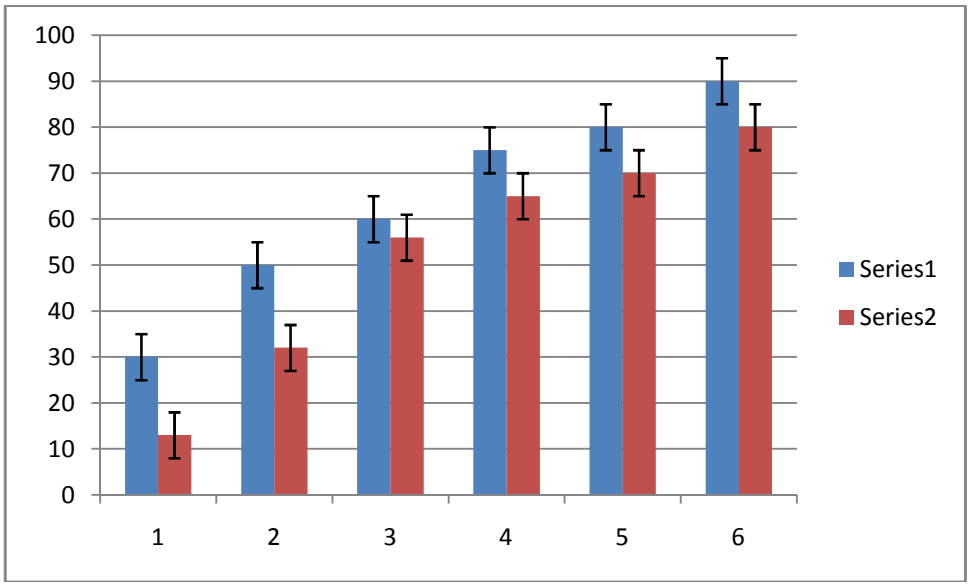
Antioxidant activity:

The antioxidant activity was compared with ascorbic acid (ASC) as standard, the IC_{50} value of formulation was found to be 62.45(Table 4). The results of estimation of the antioxidant activity of polyherbal formulations prove its action on free radicals.

Table 4: DPPH Radical Scavenging activity of Livomyn

S.NO	Concentration(µg/ml)	% Inhibition*	
		Sample	Ascorbic acid
1.	1	30.12±1.20	131.2±0.12
2.	2	50.64±0.60	32.5±0.102
3	3	60.69±1.80	56.31±0.098
4	4	75.78±1.65	65.50±0.14
5	5	80.00±1.67	70.20±0.78
6	6	90.56±1.66	80.09±0.09
	IC_{50} values	61.42	63.62

Fig 5: Graph of DPPH scavenging activity of Livomyn



Biochemical observations were further substantiated by histopathological studies. The liver sections of animals treated with CCl_4 **(Figure 6)** showed mild to marked multifocal centrilobular necrosis and diffuse granular degeneration with minimal multifocal individual cell pyknosis, mild diffuse lymphocytic infiltration. Compared to the lesions observed in the toxicant group, the lesions noted in livers of Livomyn-120 group **(Figure 8)** showed

moderately multifocal moderate centrilobular necrosis and minimal diffuse granular degeneration. Livomyn- 240 group **(Figure 9)** showed mildly multifocal minimal degree centrilobular necrosis and minimal diffuse granular degeneration, the results being almost comparable with Silymarin treated group **(Figure 7).**Livomyn-480 group **(Figure 10)** showed Multiple small foci of necrosis along with infiltration of inflammatory cells noticed, Particularly lymphocytes infiltration in the periportal region of liver. Peribillary fibrosis and bile duct hyperplasia noticed.

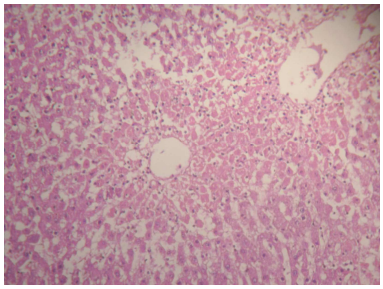


Fig-6: NORMAL CONTROL GROUP

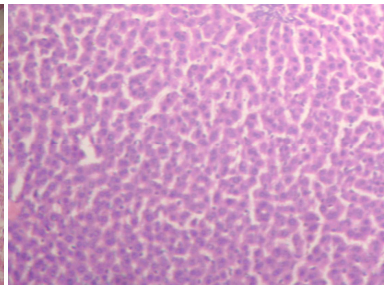


Fig-7: TOXICANT GROUP

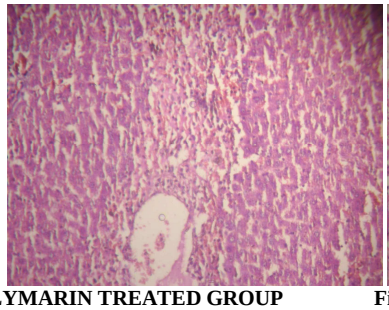


Fig-8: SILYMARIN TREATED GROUP

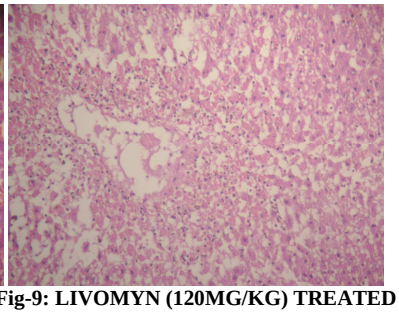


Fig-9: LIVOMYN (120MG/KG) TREATED GROUP

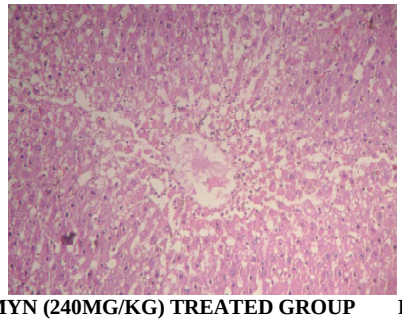


Fig-10: LIVOMYN (240MG/KG) TREATED GROUP

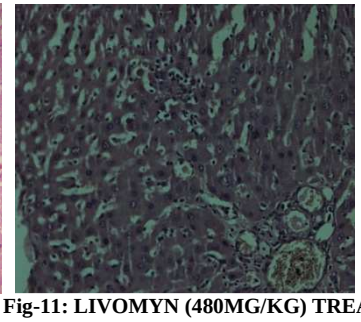


Fig-11: LIVOMYN (480MG/KG) TREATED GROUP

DISCUSSION

The efficacy of any hepatoprotective drug is essentially dependent on its capacity of either reducing the harmful effects or maintaining the normal physiologic function which has been disturbed by hepatotoxic agents [7]. CCl_4 is one of the most commonly used hepatotoxins in experimental hepatopathy; the changes associated with CCl_4-induced liver damage are similar to that of acute viral hepatitis. It is biotransformed by Cytochrome P-450 to its active metabolite, the trichloromethyl (CCl_3-) radical, which readily reacts with oxygen to form a trichloromethylperoxyl radical (CCl_3O_2-). These free radicals trigger cell damage through two mechanisms viz., covalent bonding to cellular macromolecules and peroxidative degradation of membrane lipids and endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides, which in turn yield products like malondialdehyde (MDA), which cause loss of integrity of cell membranes and damage to hepatic tissue [8]. Assessment of liver function can be performed by estimating the activity of serum enzymes SGOT, SGPT and ALP, which are enzymes originally present in high concentrations in the cytoplasm. When there is hepatic injury, these enzymes leak into the blood stream in conformity with the extent of liver damage. The elevated levels of these Marker enzymes in CCl_4-treated rats in the present study corresponded to the extensive liver damage induced by the toxin. Treatment with the test drug Livomyn as well as the reference drug silymarin significantly reduced the elevation in liver enzymes. Further, Livomyn treatment increases the levels of TP in the serum, which indicates hepatoprotective activity. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism that accelerates the regeneration process and the production of liver cells [9].

The biochemical observations were further supported by histopathological examination of liver sections of the rat. CCl_4 administration leads to centrilobular necrosis, granular degeneration with leukocyte infiltration in necrotic zone. Treatment with Livomyn at three dose levels resulted in hepatoprotection with regeneration of liver cells further confirming the hepatoprotective activity of Livomyn.

CONCLUSION

The above investigation shows that the Livomyn has promising hepatoprotective properties. The results of estimation of the antioxidant activity of polyherbal formulation, Livomyn in DPPH Radical Scavenging model has proven its action on free radicals, which indicates it's. Antioxidant activity. Further studies need to be carried out to determine other mechanisms of action that might be related to its hepatoprotective action and detailed phytochemical analysis needs to be carried out to isolate the phytoconstituents responsible for this activity.

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