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(RESEARCH ARTICLE)

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Evaluation of herbal hepatoprotective product against carbon tetrachloride induced liver problems

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Abstract

Background: When Anti-tubercular, anti-retroviral, NSAIDs, Anti-epileptic and many more drugs used on long term basis or even within therapeutic range induces hepatotoxicity. One of the chemical agent such as Carbon Tetrachloride (CCl_4) induces liver damage and leads to acute hepatotoxicity. Aims and Objectives: The present study was conducted to evaluate hepatoprotective activity of Polyherbal capsule. CCl_4 induced hepatotoxicity in wistar albino rats was studied in comparison with marketed Products. Methods: To evaluate efficacy of various products, total 7 groups of animals were studied comparatively Results: It was found that all the dosages of Polyherbal Capsule significantly reduced levels of SGOT (Serum glutamic oxaloacetic transaminase), SGPT (Serum glutamate pyruvate transaminase), Alkaline phosphatase (ALP) and Total Bilirubin when compared to CCl_4 group. There was considerable increase in total protein level in Polyherbal Capsule, Silymarin and marketed Product groups. Also all the Products tested effectively preserved the structural integrity of the hepatocellular membrane and liver cell architecture damaged by CCl_4. Conclusion: It can be concluded that Polyherbal Capsule possesses hepatoprotective activity in CCl_4 induced hepatotoxicity in rats. Polyherbal Capsule can be effectively used in hepatitis caused due to various toxins.

Keywords: Hepatoprotective; Carbon Tetrachloride; Polyherbal Capsule; SGOT; SGPT; ALP

1. Introduction

The largest organ of the human body is Liver. More than 500 metabolic functions is perform by liver. Synthesis, storage, transformation and clearance of various chemical compounds performed by liver that make it highly susceptible to injury caused by any of them. It produces a substance referred as bile this is excreted out of the body via the intestinal tract. Bile consist of various poisonous substances produced inside the metabolism that need to be timely eliminated out of the body. Thus any harm to the liver cells hampers arrangement of bile and expulsion of such poisonous substances through it. Their aggregation eventually prompts further harm to liver and entire body [1].

Hepatotoxicants or hepatotoxins are synthetic compounds that cause liver cell injury. These may will be industrial chemicals, natural chemicals, overdose of certain medicinal drugs and dietary supplements or even pesticides. A few medications may cause liver harm in any event, when utilized inside therapeutic range [2]. Hepatotoxic response is expressed in the characteristic form of cell death in Particular zones of acinar areas in liver. Liver injury in hepatotoxicity may include styles such as zonal necrosis, hepatitis, cholestasis, steatosis, granuloma, vascular lesions, neoplasm and veno-occlusive diseases. These patterns give upward push to manifestation of signs inclusive of jaundice, pruritus, severe abdominal pain, nausea, vomiting, continuous bleeding, skin rashes, generalized itching, weakness, severe fatigue, dark urine and light colored stool [3]. The accessible synthetic drugs to treat liver disorders in this condition may encourage declines the liver damage as they too need to get metabolized in already harmed liver [4]. This increments the load on liver work and desired activity of drug may not be observed. Steroids, vaccines, and antiviral drugs utilized as treatments for liver pathologies, have potential adverse side-effects, particularly if administered

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chronically or sub-chronically [5]. Thus creating pharmacologically effective agents from natural products has become a necessity by virtue of its comparatively low toxicity or fewer side effects. There are few plant derived drugs in the market which are used in liver disorders [5-8][5-8]. For the management of Hepatitis and Alcoholic Liver disease polyherbal capsule used as a hepatoprotective agent. It contains Bhumyamalaki *(Phyllanthus niruri),* Liquirice *(Glycyrrhiza glabra),* Punarnava *(Boerhaavia diffusa),* Bhringraj *(Eclipta alba),* Tulsi *(Ocimum santum),* Daruharidra *(Berberis aristata),* Pippali *(Piper longum)* extract [9-15]. Most of these extracts have shown hepatoprotective activities in isolation. The show ponder was arranged to assess hepatoprotective activity of polyherbal capsule in comparison with marketed Products in the CCl_4 induced hepatotoxicity in rats.

2. Material and methods

The study was conducted by Mprex healthcare Pune-411057. The material used for the study is given in table No.1. Institutional Animal Ethics Committee (IAEC) has approved the study in the meeting held on 30th Dec 2014.

2.1. Materials

Table 1 Materials used for the study

Drugs and Chemicals	Manufacture
Carbon tetrachloride	Merck Speciallities PVT ,Mumbai
Silymarin	Silybon (Micro Labs LTD)
Marketed Product	Himalaya Herbals

Table 2 Animals used for the study

Sr. No.	Name of animal	Weight range	Gender	Numbers to be used
1	Male Albino Wistar rats	200-250 g	Male	90

2.2. Methodology

Rats were divided into 7 cluster (n=7). Animals having a place to Group-I served as Normal control and received saline water every day for 10 days. Animals fit in to Group-II served as positive control and received only 30% CCl_4 (1 ml/kg) p.o. for 10 days. Animals from Group-III served as Standard I and received Silymarin (50mg/kg body weight) + 30% CCl_4 (1 ml/kg) p.o. for 10 days. Animals belonging to Group-IV served as Standard II and received marketed Product (100 mg/kg body weight) +30% CCl_4 (1 ml/kg) p.o. for 10 days. Animals having a place to Group-V served as Product low dose and received Product low dose (200 mg/kg body weight) + 30% CCl_4 (1 ml/kg) p.o. for 10 days. Animals in Group-VI served as Product medium dose and received Product medium dose (300 mg/kg body weight) + 30% CCl_4 (1 ml/kg) p.o. for 10 days; Animals inside Group-VII served as Product high dose and received Product high dose (400 mg/kg body weight) + 30% CCl_4 (1 ml/kg) p.o. for 10 days. All the subjects were weighed on 1st, 5th, 7th and 10th day i.e. prior to, through and later than the drug treatment. Animals were anaesthetized with light ether anesthesia. Blood was taken by retro orbital plexus puncture. Biochemical assessment of different parameters like total proteins, total Bilirubin, SGOT, SGPT, and ALP using serum were evaluated. Animals were sacrificed and liver was expelled, weighed and store in icecold saline solution for Histopathology studies.

3. Results

3.1. Effect of various Products on body weight in rats

The normal control group animals showed gradual raise in weight over the period of 10 days. Though those inebriated with CCl_4 showed considerable weight reduction during the ponder period. All other cluster, where drug is given along with CCl_4 significantly improved the body weight of animals which was near to the body weight in normal control group. When compared between the groups no measurably noteworthy distinction was noted (Figure 1).

Table 3 Effect of various Products on body weight in rats

Groups	Body Weight (Mean ± SEM)			
	1st day	**5th day**	**7th day**	**10th day**
Normal	185±2.39	185±0.81	195±1.92	199±2.10
CCl_4	165±3.12	153±0.95##	123±0.23##	105±0.97##
Silymarin	180±1.49	188±1.35**	194±1.82**	197±0.47**
Marketed Product	184±0.89	189±0.23**	193±0.67**	194±0.12**
Product Low	186±1.76	190±1.75**	196±2.23**	199±0.57**
Product Medium	178±0.56	187±0.74**	191±0.71**	196±1.89**
Product High	179±2.10	181±1.23**	189±0.54**	197±0.71**

p < 0.01 compared with the normal group, * p < 0.05, ** p < 0.01 compared with the model group

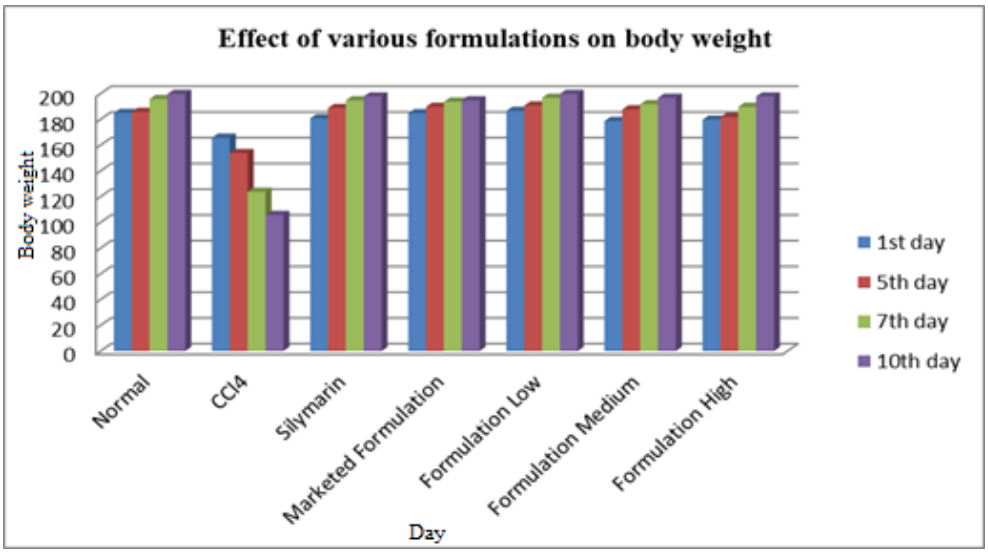

Figure 1 Effect of vario`us Products on body weight in rats

3.2. Effect of various Products on liver weight in Rats

Table 4 Effect of various Products on liver weight in Rats

Groups	Wt. of Liver in gm(Mean ± SEM)
Normal	3.61±0.05
CCl_4	4.70±0.13##
Silymarin	3.54±0.21**
Marketed Product	3.63±0.08**
Product Low	3.68±0.18**
Product Medium	3.66±0.07**
Product High	3.64±0.54**

p < 0.01 compared with the normal group, * p < 0.05, ** p < 0.01 compared with the model group

The animals in CCl_4 cluster indicated increase in liver weight when compared to that of normal group. Usually in line with the truth that CCl_4 gives rise to fatty changes in the liver that leads to increase in the liver weight. Such weight

pickup in liver is not observed with any of the Product group when compared with CCl_4 group and is near to the liver weight of normal control group.

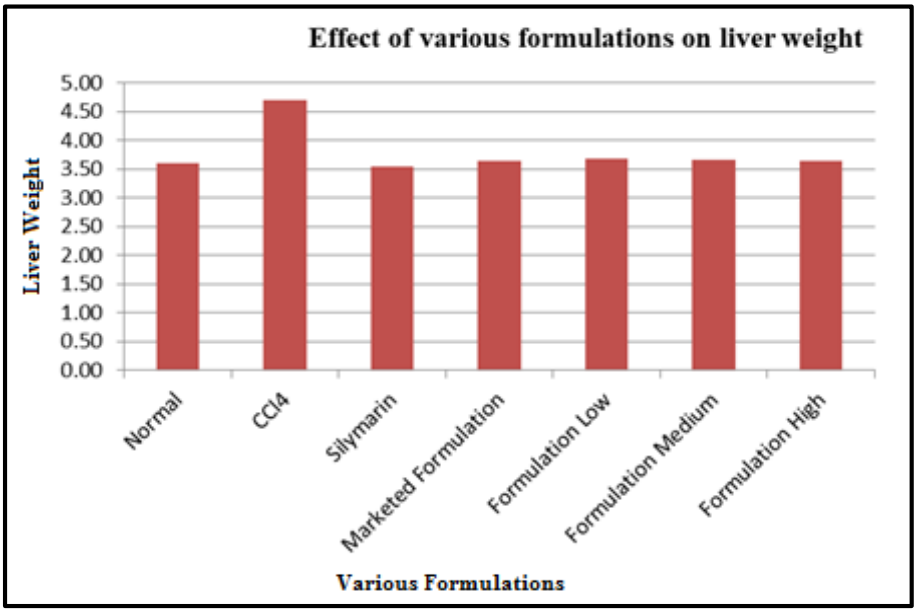

Figure 2 Effect of various Products on liver weight in Rats

3.3. Effect of various Products on SGPT, SGOT (U/L), ALP(U/L), Total Protein (g/dl), Total Bilirubin(g/dl)

Table 5 Effect of various Products on SGPT, SGOT, ALP, Total Protein, Total Bilirubin

Groups	SGPT(U/L)	SGOT(U/L)	ALP(U/L)	Total Protein (g/dl)	Total Bilirubin(g/dl)
Normal	44.32±0.56	25.8±0.38	68.19±0.15	5.76±0.01	0.88±0.08
CCL_4	87.86±0.09##	61.9±0.51##	114.50±0.15##	2.6±0.07##	4.32±0.12##
Silymarin	48.08±0.28**	26.4±0.19**	66.74±0.65**	5.78±0.12**	0.89±0.18**
Marketed Product	52.45±0.31**	30.7±0.09**	71.22±0.81**	6.47±0.08**	1.02±0.09**
Product(LD)	55.45±0.19**	32.5±0.34**	73.01±1.1**	6.41±0.13**	1.08±0.15**
Product (MD)	53.05±0.06**	30.0±0.11**	72.24±0.61**	5.95±0.04**	1.06±0.09**
Product(HD)	50.63±0.17**	27.9±0.28**	64.31±0.19**	5.47±0.06**	1.04±0.14**

$p < 0.01$ compared with the normal group, * $p < 0.05$, ** $p < 0.01$ compared with the model group

It was cleared from the outcomes of the study that CCl_4 causes considerable harm to the liver. The SGPT level in CCl_4 group was increased to 87.86±0.09, which was approximately double the level of Normal control group. All the Products significantly abridged the liver enzyme SGPT when compared to CCl_4 group. No statistical difference was observed when compared between groups. The results indicate that more than double the rise in SGOT level in CCl_4 group (61.9±0.51) when compared with normal group (25.8±0.38). All the Product significantly reduced the liver enzyme SGOT when compared to CCl_4 group. CCl_4 significantly increased ALP level (114.50±0.15) when compared with normal control group (68.19±0.15). All the Products reduced ALP level when compared to CCl_4 group. It is evident from results of the study that CCl_4 damages liver cells and impairs its function of protein synthesis. Total protein level was significantly reduced in CCl_4 group (2.60±0.07) which was half the level of normal control group (5.76±0.01). Total protein level in all other groups was increased to the level close to that of normal control group. Low dose Product and marketed Product were highly effective when compared between the groups. When compared between groups no significant difference was observed. Total Bilirubin level was increased to 4.32±0.12 g/dl in CCl_4 group. This fourfold increase compared to normal control group indicates significant liver damage due to CCl_4. All other Products significantly

reduced total bilirubin level when compared with CCl_4 group. When compared between groups there was no statistical difference.

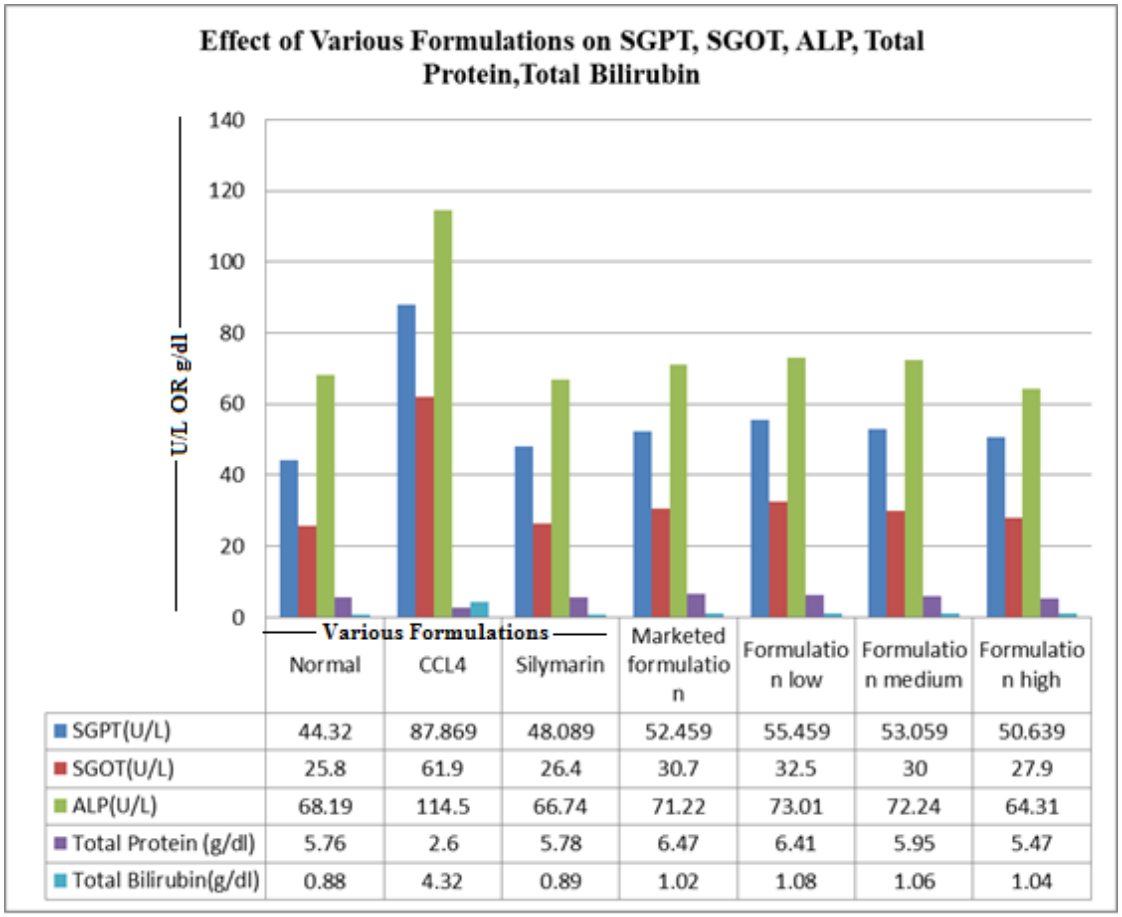

Figure 3 Effect of Various Products on SGPT, SGOT, ALP, Total Protein, Total Bilirubin

3.4. Effect of various Products on Histopathological parameters in liver

Table 6 Histopathological representation of liver in CCl_4 induced liver injury in rat treated with Various Products (H&E stain, 40X)

Groups	Necrosis	Inflammation	Blood vessels	Hemorrhage	Fibrosis	Other
Normal control	Absent	Absent	Congested	Absent	Absent	Absent
CCl_4	Present	Present	Normal	Present	Present	Fatty change
Silymarin	Absent	Absent	Normal	Absent	Absent	Fatty change
Marketed Product	Absent	Absent	Normal	Absent	Absent	Fatty change
Product Low	Absent	Absent	Normal	Present	Absent	Fatty change
Product Medium	Absent	Absent	Congested	Absent	Absent	Absent
Product High	Absent	Absent	Normal	Absent	Absent	Absent

Normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein shown by Normal control liver tissues. There were no signs of inflammation and fibrosis. Blood vessels were normal with no signs of hemorrhage. Whereas CCl_4 induced liver tissue showed signs of toxicity such as necrotic cells, inflammation, hemorrhage, fibrosis and even fatty changes. Blood vessels appeared normal. In a standard control, these signs of toxicity such as necrosis, inflammation, hemorrhage, and fibrosis were not demonstrated. But fatty changes were found and blood vessels were

normal. In the group of marketed Product, all these signs of toxicity were absent, the tissue showed normal architecture but fatty changes were noted like that of Silymarin.

In all the groups of Polyherbal Capsule i.e. low, medium and high dose, no signs of toxicity were found barring congested blood vessels in medium dose group. But this finding can also be seen in normal control liver tissue and hence it could be considered as a non-significant finding. Moreover, except the low dose Product group, liver tissues of other 2 groups were devoid of any sort of fatty changes. And therefore it can be stated that medium and high dose Product groups are better than any other groups when the histopathology of liver tissue is concerned.

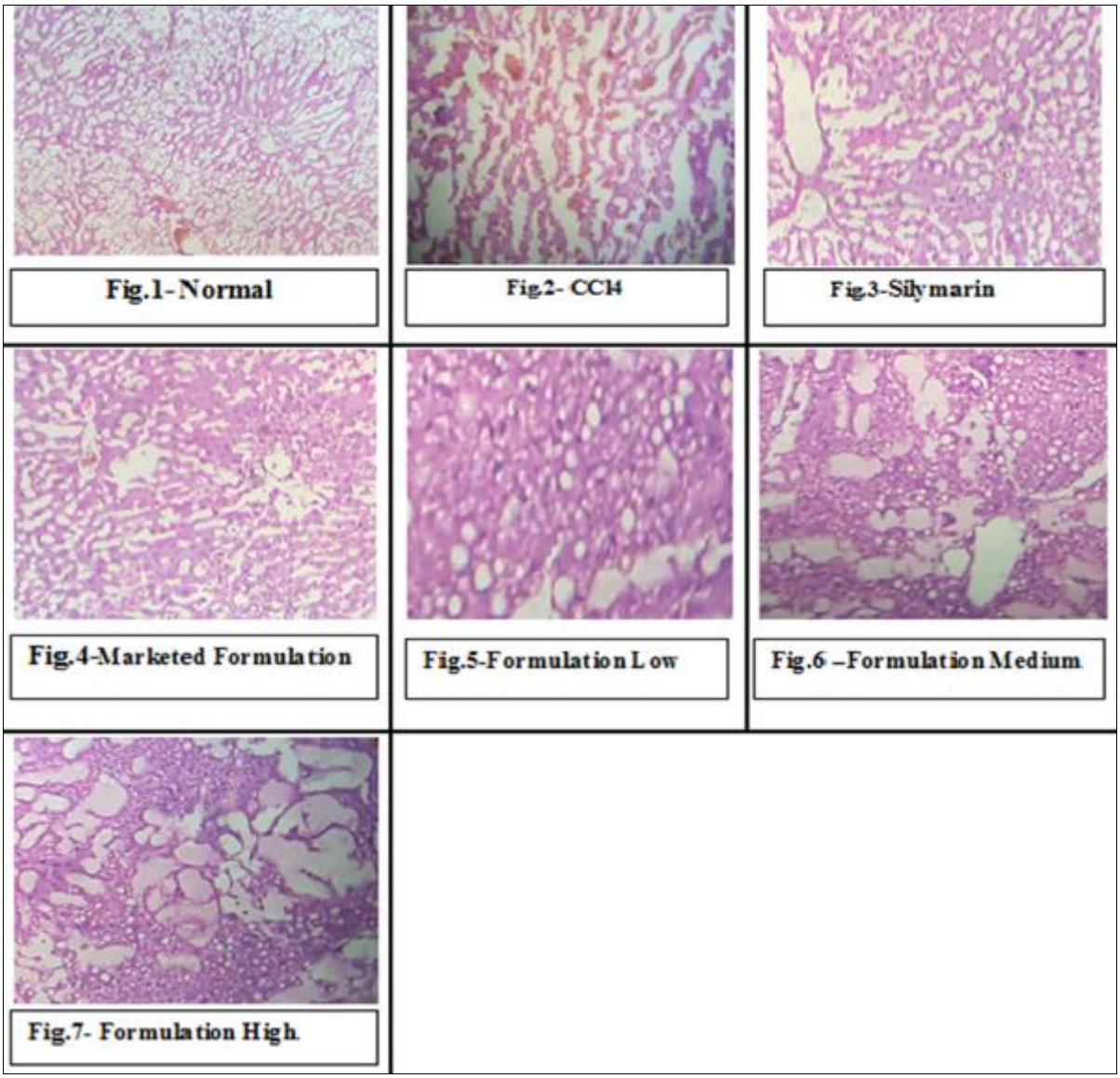

Figure 4 Histopathological representationofliver in **CCl_4** induced liver injury in rat treated with Various Product (H&Estain,40X)

4. Discussion

Within the present day period human beings are exposed to multiple chemicals directly or indirectly that will cause harm to liver cells and disable the liver work. Indications and clinically significant disease follows only when the harm leads to disability of liver function, Jaundice or yellow discoloration of skin and mucus membranes, pruritus, severe abdominal pain, nausea, vomiting, continuous bleeding, skin rashes, generalized itching, weakness, severe fatigue, dark urine and light colored stool, varicose veins, weight misfortune or weight pick up in brief period are a few of the indications that more often than not show up after disability of liver function.

Hepatotoxicity may lead to various diseases like Hepatitis, Cirrhosis, Non-alcoholic fatty liver disease, hepatocellular carcinoma, Sclerosing cholangitis and Wilson's disease. Influenced person may arrive into acute or chronic liver failure due to hepatotoxicity and subsequent liver damage [1-6].

As of now UDCA is the only allopathic drug approved by US FDA for hepatoprotection or the treatment of primary biliary cirrhosis (PBC). In spite of the fact that UDCA is reported to have hepatoprotective activity, its utilize beyond PBC is unjustified since of observed findings like liver cell failure, mutagenic effects, immune suppression, ascites, hepatitis, improved association with hepatocellular carcinoma in PBC and yet death. Moreover there is very narrow range between its therapeutic and toxic dose. Liver transplant shown in end stage kiver disease due to hepatotoxicity too carries potential unfavorable results. Graft rejection, vascular and biliary complications, sepsis, and short term survival rates are some of the confounding factors for acceptance of liver transplant. Besides its high cost and necessary ultra-modern nursing care makes it unsuitable option for general population.

Therefore, there is massive need of safer and efficient hepatoprotective agents that can prevent the damage, and improve the liver functions. In this framework, a lot of awareness has been focused on use of herbs that can provide hepatoprotective function with minimal side effects and improved acceptability. Various medicinal plants have been used in Traditional system of medicine like Ayurveda to treat jaundice and other liver disorders from thousands of years. Many of them have reported to have hepatoprotective activity and beneficial effects in liver disorders. To provide safe, cost effective, well tolerated and potential hepatprotective agent the efforts are being taken to formulate drugs from these herbs. With this background, MPREX Healthcare has conceptualized and developed Polyherbal Capsule which is a polyherbal Product of herbs with proven hepatoprotective activity. In the present study we evaluated the hepatoprotective activity of Polyherbal Capsule in comparison with Silymarin, a well-recognized hepatoprotective agent that is known to neutralize the toxic effects produced by CCl_4, acetaminophen, ethanol and galactosamine induced hepatotoxicity models in rats. Polyherbal Capsule was also compared with one more polyherbal combination present in the market for the management of liver disorders. The activity of Polyherbal Capsule was studied in three doses- high, medium and low- in three different groups. All these Products were compared with each other and with normal control and control.

All the three groups of Test Product significantly reduced the serum level of SGOT, SGPT, ALP and Total bilirubin. Whereas total Protein levels were significantly improved in treatment groups. The overall body weight was increased when compared with CCl_4 induced control group. In CCl_4 induced toxicity, resultant fatty changes in liver lead to increase in its weight. Similar results were observed in the study in CCl_4 Group. But in the groups treated with the Product no significant increase in liver weight was observed.

Liver tissues of normal control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein, when the liver tissues from all groups were subjected to histopathological examination. There were no indications of inflammation and fibrosis. Blood vessels were regular with no signs of hemorrhage. Whereas CCl_4 induced liver tissue showed signs of toxicity as necrotic cells, inflammation, hemorrhage, fibrosis and even fatty changes. Blood vessels appeared normal. In a standard control, these signs of toxicity such as necrosis, inflammation, hemorrhage, and fibrosis were not demonstrated. But fatty changes were found and blood vessels were normal. The signs of toxicity were absent in the group of marketed product, the tissue showed normal structure but fatty changes were seen like that of Silymarin.

In all the groups of Polyherbal Capsule i.e. low dose to high dose, no signs of toxicity were found excepting congested blood vessels in medium dose group. But this finding can also be seen in normal control liver tissue and hence it could be considered as a non-significant finding. Moreover, except the low dose Product group, liver tissues of other 2 groups were devoid of any sort of fatty changes. And therefore it can be stated that medium and high dose Product groups are better than any other group in comparison when the histopathology of liver tissue is concerned.

All the above findings suggest that high, medium and low dose Product groups show significant hepatoprotective activity in terms of reduction in serum levels of liver enzymes such as SGOT, SGPT, ALP that are usually released in response to the damage to hepatic parenchyma. Lowering of Total bilirubin may lead to improvement in Liver function. All these effects are highly significant when compared with CCl_4 group, establishing its heaptoprotective activity [7-15].

5. Conclusion

It can be concluded from the results of the present study that Polyherbal Capsule possesses hepatoprotective activity in high, medium and low doses in CCl_4 induced hepatotoxicity in rats. No signs and symptoms of toxicity in any group were observed and all the Products tested were well tolerated by the rats. Though high dose and low dose Products are

effective as hepatoprotective agents, medium dose Product of Polyherbal Capsule is recommended for potent hepatoprotective effect in humans.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

I declare no conflict of interest.

Statement of ethical approval

All the experiments were permitted and conducted as per the guidelines of Institutional Animal Ethical Committee (Approval no. CPCSEA/IAEC- 009/2022).

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