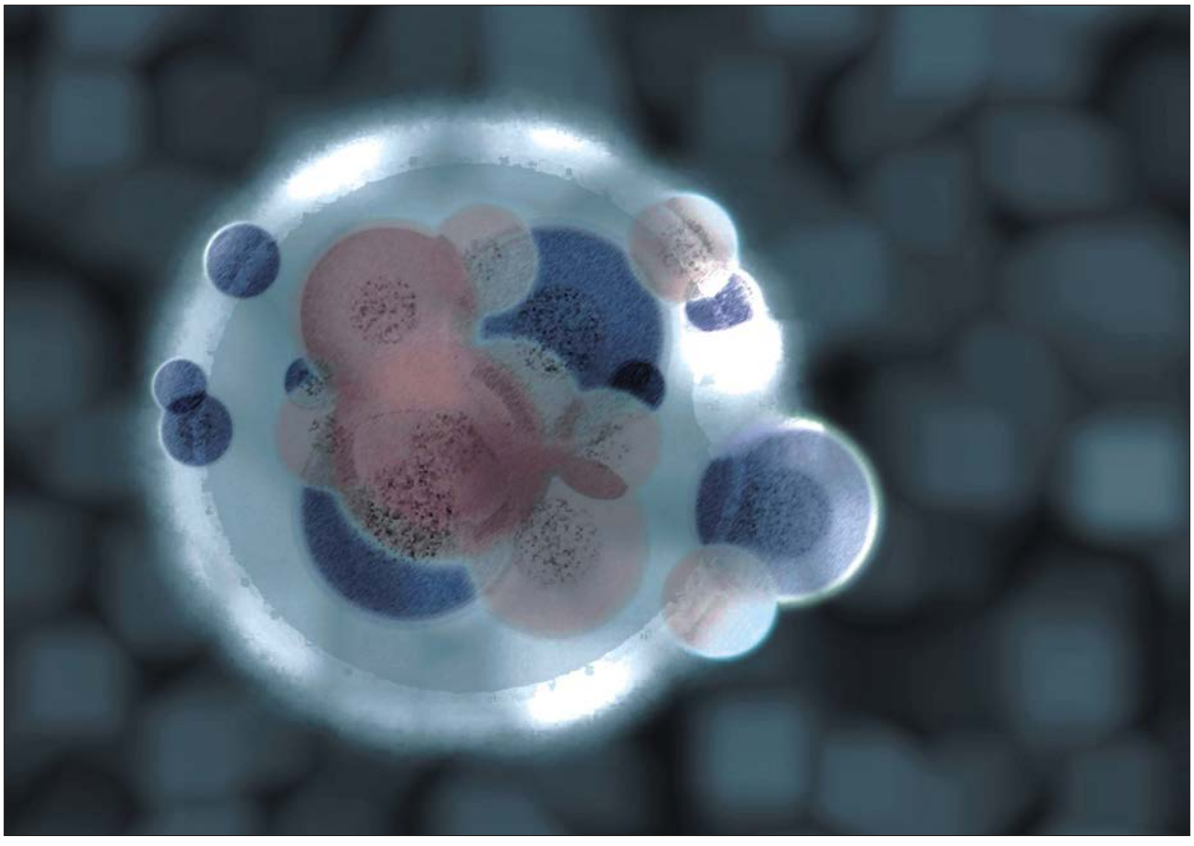

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Hydroalcoholic Extract of *Terminalia arjuna*: A Potential Hepatoprotective Herb

[1]U. Subasini, [1]G.V. Rajamanickam, [1]G.P. Dubey, [1]P.C. Prabu, [1]C. Savariraj Sahayam, [1]M. Mohammed Shabi, [1]K. Gayathri and [2]Aruna Agrawal
[1]Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Thanjavur-613 402, India
[2]Department of Basic Principles, Institute of Medical Sciences and Center for Psychosomatic and Biofeedback Medicine, Banaras Hindu University, 221 005, India

Abstract: Herbal drugs are considered to be the best alternative for many diseases. Even incurable diseases like cancer, HIV, etc., are moving towards the traditional herbal drugs. The treatment using herbal drugs is gaining momentum to the vital organs like liver, kidney and heart. In such movement, the finding of the presence of effectiveness of hepatoprotective factor from any plant is felt a contributory one. *Terminalia arjuna* a plant commonly observed in the south India is found to have a property of hepatoprotectiveness. In order to appreciate the hepatoprotective activity of the *Terminalia arjuna*, hydroalcoholic extract of the same is used here on Albino Wistar mice. The general behaviour of the animals has been studied in comparison to control group with the *Terminalia arjuna* extract treated animals. A gross pathology and histopathology have also been studied. The results obtained from the study of GOT, GPT and ALP clearly direct the hepatoprotective effectiveness of hydroalcoholic extract of *Terminalia arjuna*.

Key words: Hepatoprotective, *Terminalia arjuna*, CCl_4, histopathology, haematological parameters, necropsy

INTRODUCTION

Liver is a vital organ among the vertebrate bodies. Even the heart and kidneys are now-a-days being transplanted, but the transplantation of liver is still at large. The advanced civilization and luxury in life are bringing a lot of testing and trials to our body in the form of pollution. Even the innocent human beings without taking toxic substances in the food or water, their blood contents are enriched with pollutants like lead, arsenic, cadmium and mercury through the inhalation of polluted atmosphere, triggered by the industrial exhaust, cosmetics, automobiles, etc. (Bray and Bettger, 1990; Williams, 1984; Muriel *et al*., 2001).

After the publication of the presence of excess heavy metals in the Ayurvedic drugs in the JAMA article (Saper *et al*., 2004), the importance given to Ayurvedic herbal drugs as alternative medicine has been brought to a question mark. The ban order on Ayurvedic products either in the form of pharmaceuticals or neutraceuticals has surrogated the popularity of Ayurvedic drugs. Under such circumstances, it is very essential to protect the traditional system particularly herbal medicines. The results of such testing have tarnished the validity of herbal drugs.

Keeping this in mind, the herbal plants here, are exposed for such studies. Such efforts have already been taken by many researchers on plants like *Spirulina maxima*, *Picrorhiza kurroa*, *Eclipta alba*, *Boehemeia nieva*, *Cichurium intybus*, etc. (Anandan *et al*., 1999). In spite of confirmatory results in the hepatoprotective effects for the above mentioned plants, there are still many more herbal plants which have to be studied for the hepatoprotective effects. Once the question on the veracity of herbal medicine is brought forward, it is felt that without any further delay such work has to be expedited. With that aim, the plant *Terminalia arjuna* has been taken up here for the research work.

Terminalia arjuna is an ornamental tree as well as a tree of shade. It is also known as an adaptogen. The bark of this tree has been commonly used in Ayurvedic preparations to bring hepatoprotective effect in a drug. It is considered to be a common plant in the lower Himalayas. However, in the southern tip of Tamil Nadu, it is found in the coastal ridges. While scanning the literature, a scientific approach using this plant for

Corresponding Author: Dr. G. Victor Rajamanickam, Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Thanjavur-613 402, India
Tel: 04362-264346/304116 Fax: 04362-264346/264120

understanding the hepatoprotective effect could not be seen but for a few papers which have adopted either as antioxidant (Gupta *et al*., 2001) or powerful cardiotonic (Karthikeyan *et al*., 2003) or the salutary effect (Bharani *et al*., 1995). The scientific studies using *Terminalia arjuna* for the natural treatment has been effected by either aqueous extract or 50% ethyl alcohol has been studied. In spite of the efforts made in understanding the hepatoprotective effects from *Terminalia arjuna*, hydroalcoholic extract has not been applied so far. Under such status, it is felt that a study using hydroalcoholic extract against the hepatotoxin CCl_4 may throw better light on the hepatoprotective effect from the plant *Terminalia arjuna.*

While screening the literature, it is observed that a number of pharmacological and chemical agents act as hepatotoxin and produce variety of liver ailments (Liv *et al*., 1994). Among them, carbon tetrachloride intoxication in mice is an experimental model widely used for necrotic and steatotic changes in hepatic tissue (Parola *et al*., 1992). The study on hepatoprotective effect using CCl_4, for any medicinal plant's extraction will be of great interest and significant in standardizing the formulation and declare globally, the validity of the herbal products (Mehmet Kanter *et al*., 2005; Nagi *et al*., 1999; Al-Gharably *et al*., 1997).

MATERIALS AND METHODS

Plant materials: Bark of *Terminalia arjuna* (TA) was collected from Tirunelveli, Tamil Nadu, India. The plant was previously identified and authenticated by experts in the Rabinat Herbarium, St.Joseph College, Trichy, St. Xavier's College, Palayamkottai and Botanical Survey, CCRAS Unit, Govt. Medical College, Palayamkottai. The collected materials were washed thoroughly in water, chopped and kept for air drying. Then, the plants were shade dried for nearly 15 days. Once dried, they were crushed to get coarse powder.

The pulverized plant materials were taken up for extraction using Hydro Alcoholic Extract (HAE) in the proportion of 30:70. The extraction was carried out by cold percolation method. The extracts were then dried in vaccuo and they were stored in desiccators and kept in refrigerator for further use.

Experimental animals: Twenty-four male and female Albino Mice of Wister strain weighing 25-35 g were taken from SASTRA Animal House, Thanjavur. The animals were housed in polypropylene cages and maintained in controlled temperature (22±2°C) and 12 h light and dark cycle. They were fed with the normal rodent feed supplied by Venkateswara Animal House, Bangalore. Water was supplied *ad libitum*. They were given a week's time to get acclimatized with the laboratory conditions. Initial body weight of each animal was recorded. Ethical clearance for the use of animals was obtained from the committee constituted for the purpose in SASTRA.

Experimental induction of hepatic damage: Liver damage was induced in mice by administering CCl_4 in a form of suspension of Liquid Paraffin (LP) in the ratio of 3% V/V at the dose of 1 mL CCl_4/kg body weight of each animal by adopting the route of intra-peritoneal (ip) in the lower abdomen (Rajesh and Latha, 2001; Liv *et al*., 1994). CCl_4 was administered twice a week on every first and fourth day of all the 22 days.

Experimental design: Mice were divided into 6 groups of 6 animals each as revealed in OECD guidelines (OECD, 2000).

Group I animals served as control and given ip administration of LP only at the dose of 3 mL kg^{-1} b.w. Group II animals constituted hepatotoxic mice and provided ip administration of LP+CCl_4 twice a week for 22 days. Group III animals were treated with standard drug of silymarin (25 mg kg^{-1} bw) + Ccl_4. Group IV, V and VI animals were the herb-treated animals and given oral administration of *Terminalia arjuna*. After treating animals with drug at different doses of 50, 100, 200 mg kg^{-1} for 21 days CCl_4 was administered.

A known quantity of fresh food daily at 9.30 am was provided. Thereby, measuring the difference in the food intake in a day, the daily food consumption of the previous day was carried out. Once the measurement of food intake is over, the LP, LP+CCl_4, daily food consumption Std+CCl_4, LP+CCl_4+TA were administered at the same time between 10-11 am. Body weights of Mice were recorded weekly to assess percentage of weight gained in each group. General well being and behaviour of the animals were observed daily throughout the period of study. The lither in the cage was renewed twice a week to ensure maximum comfort for the animals.

Animals were kept starved overnight on the 21st day. On the next day, after recording the weight in each case, they were sacrificed by decapitation and making an incision on jugular vein to collect blood. The liver, heart, kidney, spleen and testes were dissected out, blotted off blood, washed in saline and weighed instantaneously. This was kept in frozen containers and proceeded to biochemical estimations.

Biochemical parameters: Plasma was prepared from the collected blood and subjected to biochemical estimation of different hepatic markers such as GOT (Rellman and

Table 1: Qualitative observation of macroscopic characters

S. No.	Group	Apparently normal hepatocytes	Vacuolar degene-ration	Spotty necrosis	MNC infiltration	Diffuse necrosis
1	I	*	-	-	-	-
2	I	*	-	-	-	-
3	I	*	*	-	*	-
4	II	-	*	*	*	*
5	II	-	*	*	*	*
6	III	-	*	*	*	*
7	III	-	*	*	*	*
8	III	-	*	-	*	*
9	III	-	*	*	*	*
10	IV	*	*	*	*	-
11	IV	*	*	*	*	-
12	V	*	*	*	*	-
13	V	*	*	*	*	-
14	V	*	*	*	*	-
15	VI	*	*	*	*	-
16	VI	*	*	*	*	-
17	VI	*	*	*	*	-

-: Absent, *$p<0.05$

Frankel, 1957), GPT (Rellman and Frankel, 1957) ALP (King and King, 1954), Protein (Lowry *et al*., 1951) and Thio barbituric acid reactive substances (TBARS) (Yagi *et al*., 1979), Reduced glutathione (Beutler *et al*., 1963). 10% liver homogenate in Tris HCL buffer of 0.1 M (pH 7.4) was also used for the estimation of enzymes.

Histopathology: Gross pathology of animal organs has been macroscopically examined and the observed characters are qualitatively tabulated (Table 1). A portion of liver tissue in each group was fixed in 10% formalsal (formalin diluted to 10% with normal saline.) and proceeded to histopathology. After paraffin embedding and block making, several sections were made, stained with Haematoxylin and Eosin and examined under microscope. A few photomicrographs of representative types were also taken.

Statistical analysis: Statistical analysis was carried out using student t-test and the values of mean and ±SD.

RESULTS

Food consumption and weight gain: We observed that food consumption and weight gained significantly increased in group IV, V and VI as compared to other groups. In group II mice, there was a lesser weight gain as compared to group I animals (Table 2).

Animal organ weight: Organs like liver and kidney were found to be increased in disease control groups. But, on herb extract treated animals decrease in weight of organs like liver and kidney was observed (Table 3).

Haematological parameters: Hematological parameters like Haemoglobin, Erythrocytes and Leucocytes were observed in both control and treated groups. Haemoglobin and erythrocytes level were found to decrease on CCl_4 induced mice, whereas in the herb extract treated animals, the Haemoglobin was found to increase in a dose dependent manner. At the same time, the leucocytes were seen to increase in CCl_4 induced mice. But, in herb extract treated animals a decrease in a dose dependent manner was noticed (Table 4)

Table 2: Observation of change in body weight (values are mean±SEM)

Groups	Body weight (g) Before treatment	After treatment
Normal	31.49±2.4	33.76±2.3
Disease control	32.23±2.4	29.97±2.7
Standard drug	31.88±2.5	32.94±2.2
TA1	32.22±2.3	33.20±2.3
TA2	32.81±2.5	34.22±2.6
TA3	32.05±1.7	34.80±1.01

Hepatic marker enzymes: Hepatic marker enzymes like ALP, GOT and GPT were found to go high to a greater extent in plasma to those animals treated with CCl_4. But, in the case of TA herb extract treated animals dose dependent increase in activity of those enzymes could be accounted significantly (Athar *et al*., 1997). The level of these enzymes in hepatic tissue was found to decrease in disease control groups, whereas, in those herb extract treated animals, the level of these enzymes were noticed to increase and $p<0.05$ significant difference was noted at the highest dose of *Terminalia arjuna* extract treatment. Though the effect is very low when compared to standard drug treatment group, the results indicate that the extract is able to exhibit hepatoprotective activity to some extent (Table 5).

Antioxidant study: The level of reduced Glutathione in CCl_4 treated mice were observed to be high while at the same time, the TA herb extract treated animals also indicated a significant raise in glutathione level (Anandan *et al*., 1999). Instead, the thio barbuturic acid (TBARS) level in hepatic cell of those animals treated with CCl_4 though displayed an increase in values, unlike the above case, the values of TBARS in the case TA herb extract treated animals, were decreased (Table 6). This study reveals that *Terminalia arjuna* is also antioxidant in nature.

The levels of protein in plasma and hepatic tissue were much decreased in CCl_4 treated animals. It was the other way in the case of TA herb extract treated animals. However, the same swang to normal level at higher dose of extract.

Gross pathology/necropsy report

Group I (1, 2, 3): Livers of all the three mice showed no gross pathological changes.

Table 3: Observation of changes in organ weight (values are mean±SEM)

Groups	Liver (g)	Heart (g)	Kidney (g)	Spleen (g)	Testis (g)
Normal	1.17±0.08	0.16±0.00	0.46±0.05	0.46±0.25	0.26±0.01
Disease control	2.21±0.13	0.14±0.00	0.57±0.03	0.14±0.03	0.18±0.07
Standard drug	1.36±0.13	0.16±0.001	0.54±0.07	0.43±0.18	0.22±0.05
TA1	1.56±0.03	0.13±0.00	0.36±0.01	0.12±0.00	0.15±0.02
TA2	1.49±0.08	0.14±0.00	0.45±0.08	0.11±0.00	0.17±0.02
TA3	1.56±0.05	0.12±0.00	0.41±0.07	0.12±0.00	0.17±0.01

Table 4: Observation of changes in haematological parameters (values are mean±SEM)

Groups	Hb (mg dL^{-1})	RBC (Million cells $cu.mm^{-1}$ of blood)	WBC (Cells $cu.mm^{-1}$ of blood)
Normal	11.6±0.21	3.30±0.17	7956.00±364.42
Disease control	8.9±0.12	2.77±0.06	9434.25±266.92
Standard drug	11.4±0.21	3.45±0.17	8050.00±322.67
TA1	10.6±0.15	2.50±0.13	7101.50±230.13
TA2	11.4±0.18	3.00±0.16	8223.50±578.56
TA3	11.32±0.19	3.18±0.30	7730.75±125.18

Table 5: Effect of standard drug and *Terminalia arjuna* on the activity of plasma and hepatic enzymes (values are mean±SD)

Groups	GOT level in plasma (U L^{-1})	GPT level in plasma (U L^{-1})	ALP level in plasma (KA U^{-1})	GOT level in liver (μg mg^{-1} min^{-1} of pyruvate liberated in protein)	GPT level in liver (μg mg^{-1} min^{-1} of pyruvate liberated in protein)	ALP (μg mg^{-1} min^{-1} of phenol liberated in protein)
Normal	101.0±3.5	120.5±2.3	12.05±0.3	2.3±0.01	2.7±0.01	1.3±0.01
Disease control	391.2±5.2**	420.2±6.3**	90.0±0.6**	1.4±0.02*	1.2±0.02*	0.5±0.01*
TA 1	351.0±3.6	325.3±2.5	79.3±0.7	1.6±0.03	1.6±0.01	0.6±0.02
TA 2	252.5±4.2	290.4±3.2	70.5±0.6*	2.1±0.01	2.0±0.02	0.9±0.01
TA 3	190.5±5.3*	210.5±5.3*	59.3±0.5**	2.4±0.02*	2.3±0.03*	1.2±0.02*
Standard	150.2±1.2**	200.2±3.5*	55.4±0.4**	2.5±0.01*	2.2±0.01*	1.5±0.01*

*p<0.05, **p<0.01

Table 6: Effect of standard drug and *Terminalia arjuna* on the activity of plasma and hepatic protein, hepatic reduced glutathione and TBARS level (values are mean±SD)

Groups	Plasma protein (g dL^{-1})	Liver protein (g $100\ g^{-1}$)	Reduced glutathione (μg g^{-1} of GSH in liver)	TBARS (n mol g^{-1} of MDA in liver)
Normal	7.5±0.5	7.9±0.3	2916.0±12.3	115.3±2.3
Disease control	5.3±0.6*	4.9±0.6*	1147.1±13.2*	330.2±6.5*
TA 1	5.9±0.6	5.5±0.2	1723.02±14.5	390.6±5.6
TA 2	6.4±0.5	6.9±0.4	2102.05±15.2	216.5±6.9
TA 3	7.2±0.4*	7.8±0.3*	2245.3±19.6	195.3±6.3
Standard	7.0±0.5*	8.0±0.4*	2612.32±16.5*	160.2±8.9*

*p<0.05, **p< 0.01

Group II (4, 5): Liver of mouse no: 4 reflected patchy areas of necrosis throughout the external surface. The mouse no: 5 indicated a pale diffuse of discolouration of liver.

Group III (6, 7, 8, 9): While the Livers of mice 6, 8 and 9 were apparently normal, the mouse no: 7 registered a very pale discolouration.

Group IV (10, 11): Only mouse no: 11 indicated relatively pale colour, while the other one was normal.

Group V (12, 13 and 14): No gross pathological changes were observed except for a pale discolouration of liver in mouse no: 13.

Group VI (15, 16 and 17): Pale focal areas of discolouration were present on the dorsal surface of the liver of mice no: 16. No gross changes were detected in all other mice.

Histopathology report

Group I (1, 2, 3): The liver sections of all the three mice revealed normal hepatocytes and mild to moderate congestion of blood vessels which correlated with the gross findings (Fig. 1).

Group II (4, 5): Severe colliquative necrosis of the hepatocytes especially in the peripheral areas of the most of the portal triads i.e., the periportal hepatocytes was consistently observed in the sections of both the mice. There were large vacuolated spaces in the areas of necrosis where the necrotic debris had been completely phagocytosed. Massive mononuclear cell infiltration was consistently observed. Around 50-60% of the liver parenchyma was found to be necrosed. The histopathological findings correlated with the necrotic patches observed during necropsy (Fig. 2, 5 and 6).

Group III (6, 7, 8, 9): The liver sections revealed diffuse colliquative necrosis along with severe mononuclear cell

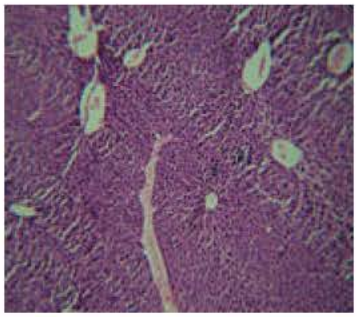

Fig. 1: Normal liver section showing apparently normal hepatocytes (Group I-Control) 10X

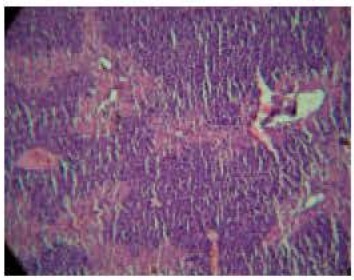

Fig. 2: CCl_4 induced hepatotoxicity. Note areas of diffuse necrosis (arrow) around the portal triads (Group II) 10X

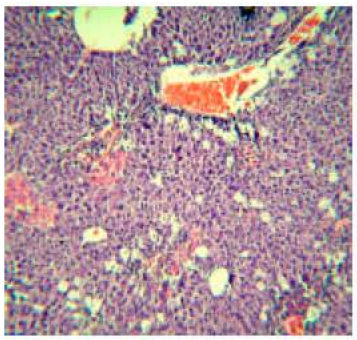

Fig. 3: Liver of CCl_4 treated mice with periportal necrosis of hepatocytes and haemorrhages (arrow) (Group III) 10X

infiltration. The necrosed hepatocytes were having intensely eosinophilic cytoplasm with nuclear remnants, seen as karyorhectic fragments. The area involved was relatively less than that of the group II (Fig. 3 and 4).

Group IV (10, 11): Moderate hydropic degeneration along with very few areas of spotty necrosis was observed consistently in both the sections. There were no areas of diffuse necrosis as observed in the groups II and III (Fig. 7).

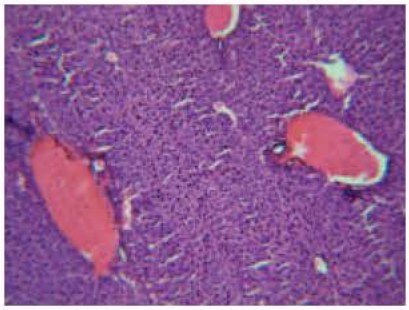

Fig. 4: TA_1 treated liver section revealing apparently normal hepatocytes with engorged blood vessels. (Group III) 10X

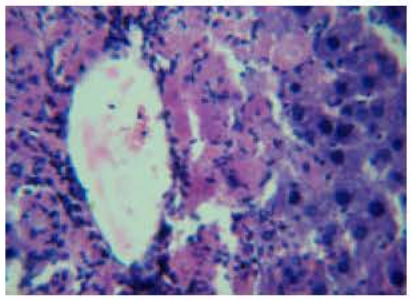

Fig. 5: Necrotic hepatocytes around the blood vessels with intensely eosinophilic cytoplasm and karyolytic/pyknotic nuclei (Group II) 50X

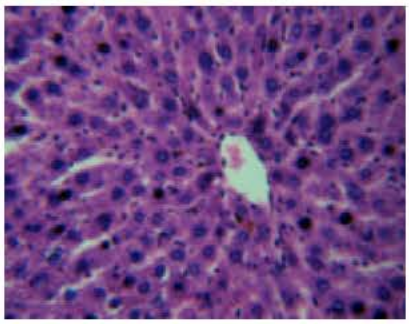

Fig. 6: Apparently normal hepatocytes of TA_2 treated mice. Note the presence of mild hepatocytes swelling and thin sinusoidal spaces (arrow) (Group II) 50X

Group V (12, 13 and 14): The hepatocytes were near normal with a few areas of spotty necrosis. No diffuse necrosis was observed (Fig. 8).

Group VI (15, 16 and 17): Focal areas of hydropic changes were observed throughout the sections along

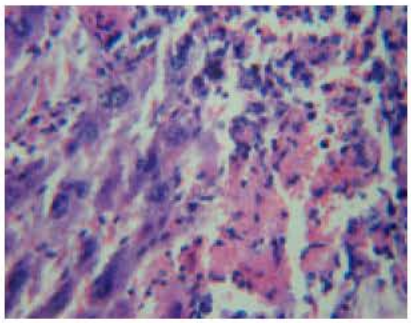

Fig. 7: Intense necrosis (arrow) along with haemorrhages in CCl_4 treated mice liver section (Group IV) 50X

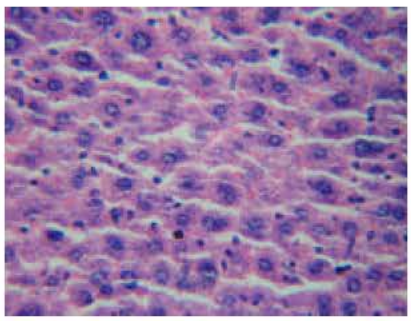

Fig. 8: TA_3 treated mice liver with normal hepatocytes and sinusoids (Group V) 50X

with few areas of mononuclear infiltration and occasional spotty necrosis. No diffuse necrotic changes were observed in all the three sections.

DISCUSSION

Recknagel (1967) has established the CCl_4 metabolization in the liver by the highly reactive trichloromethyl radical and auto-oxidation of the fatty acids present in the cytoplasmic membrane phospholipids by the free radical made available by CCl_4 metabolisation and functional and morphological changes taking place in the cell membrane. The same has been attested due to the elevation of marker enzymes like GOT, GPT and ALP in serum. Above all, they have also proved the hepatoprotectivity against CCl_4 induced liver damages by using a polyherbal formulation, known as Himoliv which is a combination of 25 different herbal plants (Bhattacharyya *et al*., 2003a, b). However, in those 25 plants they have not included the *Terminalia arjuna* which is taken up for the present study. Here, it is noticed that *Terminalia arjuna* is producing a better hepatoprotective activity against CCl_4 induced liver damages.

On induction of disease with CCl_4 GOT, GPT and ALP level in plasma were found to be increased significantly ($p<0.01$) and it was found to be 3.87, 3.48, 7.5 times, respectively when compared to the normal animals. But on *Terminalia arjuna* treated animals at the doses of 50, 100, 200 mg kg^{-1} bw, a decrease in the enzyme level according to the dosage levels has been observed. When compared to the control group, in the case of GOT, the level in plasma is found to be 3.5, 2.5 and 1.9 times, respectively while GPT level remains to be of 2.7, 2.4 and 1.8 times and ALP level of 6.3, 5.6 and 4.9 times at 50, 100 and 200 mg kg^{-1} bw, respectively. It has been noticed that the different levels of enzyme changes are in very close range in activities to the standard modern drug silymarin, which is also bringing a decrease in the level of enzyme in plasma like GOT by 1.4 times, GPT by 1.7 times and ALP by 4.6 times.

Instead of the serum plasma when the homogenated livers of both CCl_4 inducted and extract treated are examined GOT, GPT and ALP levels in liver is found to be decreased by 0.6, 0.4 and 0.4 times, respectively. But, for *Termianlia arjuna* extract treated animals keep the variation in the enzyme level according to the dose rate such as 50, 100 and 200 mg kg^{-1} b. w. As revealed from the percentage values of GOT from 14 to 71%, GPT from 33.3 to 91.7% and ALP 20 to 40%, it is suggested that the extract provides due protection from liver damage. In other words, it enables the prevention of liver damage. The observed values of GOT, GPT and ALP in *Terminalia arjuna* extract treated cases are comparable to the standard drug silymarin values in such a way silymarin provides a preventive level to the tune of GOT by 61.6% while GPT by 52.4% and ALP by 38.4%.

Likewise, in the protein levels of plasma and liver, are found to be decreased significantly in the CCl_4 inducted animals ($p<0.01$) whereas the *Terminalia arjuna* extract treated, it is noticed to have increase to the tune of the normal level at 200 mg kg^{-1} bw. Hypo-albuminaemia is the most frequently observed one in advanced chronic liver diseases. Hence, decline in total protein content can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases (Venukumar *et al*., 2002). The lowered level of total proteins recorded here in the plasma as well as liver of CCl_4 inducted mice reveals the severity of hepatopathy. The attainment of near normalcy in total protein contents of both plasma and liver of herb extract-treated mice further, attests the hepatoprotective effect of *Terminalia arjuna*.

It is well known that the hepatotoxic effect of carbon tetrachloride is due to the oxidative damage by free radical generation and antioxidant property is claimed to be one of the mechanisms of hepatoprotective drugs (Pandit *et al*., 2004).

Malondialdehyde level in liver is found to increase very rapidly to a significant level of $p<0.05$. On *Terminalia arjuna* treated animals the level of malondialdehyde is observed to decrease. Though significant difference is not noted in any dose when compared to the disease control animals, the extract treated is found to reflect a decrease to some extent. At 200 mg kg^{-1} bw of extract treated case, malondialdehyde level is found to increase by 1.6 times but in disease condition, it is seen to have an increase by 2.9 times when compared to the normal animals. But, Silymarin drug is able to exhibit significant difference $p<0.05$ when compared to the disease control animals.

Liver is the major site for the synthesis of GSH (Parola *et al*., 1993). The detoxification of different drugs and xenobiotics in the liver interferes with GSH (Seven *et al*., 2004). It was found to be decreased significantly by $p<0.05$. No significant difference was found on treating animals with different doses like 50, 100, 200 mg kg^{-1} b. w. But the extract was found to be able to exhibit some percent of protection in liver by increasing the level of GSH by 1.95 times higher than the disease control animals. But on silymarin treated animals reduced glutathione level is recorded to have a significant increase in comparison to the disease control animal. As suggested by Prasenjit Manna *et al*. (2006) in their studies on aqueous extract of *Terminalia arjuna*, here too, decrease in Reduced Glutathione level in liver of CCL_4 toxicity is attributed to the decreased availability of GSH, resulted during the enhanced lipid peroxidation.

Lu (1999) has put forth that the decreased hepatic GSH in CCl_4 intoxicated mice could be as a result of Hexose Monophosphate (HMP) which shunts the impairment due to CCL_4 intoxication. Thereby, NADPH availability is reduced and the ability to recycle GSSG in GSH is decreased. Likewise, it is surmised that *Termianlia arjuna* extract may exhibit its activity by blocking oxidative damage through lipid peroxidation and protein oxidation which must have enabled the prevention of the loss of membrane permeability and dysfunction of cellular proteins and in turn, a decrease in the endogenous level of hydroxyl radical.

Noguchi *et al*. (1982) have observed the metabolisation of CCl_4 in the liver by the cytochrome P450-dependent monooxygenase systems followed by its conversion to more chemically active form, trichloromethyl radical. Rajesh and Latha (2004) explained that the enzymes involved in this process are located in the endoplasmic reticulum of the liver and their activities are dependent on many environmental factors. Prasenjit Manna *et al*. (2006) have attributed the cause of metabolisation CCl_4 to cytochrome P450 and other environmental factors to the results obtained for the aqueous extract of *Terminalia arjuna*. In the present case too, it is presumed that hepatoprotective and antioxidant activity of the hydroalcoholic extract of *Terminalia arjuna* must have been the result of what Prasenjit Manna *et al*. (2006) has proposed. However, the nature of this extract being different to the above one, the enzymes of endoplasmic reticulum involved in trichloromethyl radical formation has to be evaluated for confirmation.

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