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Research article

HEPATOPROTECTIVE ACTIVITY OF A UNANI POLYHERBAL FORMULATION "KABIDEEN" IN CCl_4 INDUCED LIVER TOXICITY IN RATS

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ABSTRACT

Objective: To evaluate the hepatoprotective effect of Kabideen Syrup in rats treated with carbon tetrachloride.

Methods: In hepatotoxic rats, liver damage was studied by assessing parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, total proteins, and cholesterol and lipid peroxidation. Histopathological study of the liver in experimental animals was also undertaken.

Results: Hepatic damage as evidenced by a rise in the levels of AST, ALT, ALP, bilirubin cholesterol and Malondialdehyde (MDA) and decreased level of total protein in serum. Liver showed a tendency to attain near normalcy in animals co-administered with kabideen (50 ml/kg) significantly reduced the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and cholesterol levels and increased the total protein levels when compared to the CCl4 group. The histopathological findings showed a significant difference between the Kabideen (50ml/kg) and CCL4 treated groups.

Conclusion: The study substantiates the hepatoprotective potential of Kabideen.

Key words: Carbon tetrachloride, marker enzymes, cholesterol, total protein.

INTRODUCTION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. It has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways responsible for growth, fight against disease, nutrient supply, energy provision and reproduction. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, blood coagulation, immunomodulation, secretion of bile and storage of vitamins. Liver damage inflicted by hepatotoxic agents is of grave consequences. Liver ailments represent a major global health problem. (Subramonian, A., Pushpangadan, P, 1999).

Inspite of the tremendous advances in modern medicine, no effective drugs are available, which stimulate liver functions and/or offer protection to the liver from damage or help to regenerate hepatic cells (Sharma, S. K et al, 2000). Polyherbal preparations are used for the treatment of various diseases by the local medical practitioners all over India (Lal, A. S et al, 2007). Many Polyherbal formulations whether pharmacopoeial or nonpharmacopoeial are used in abundance in the markets, but most of them have still not been scientifically evaluated for their described hepatoprotective and related effects. *Kabideen* is one such compound formulation manufactured by a reputed Unani pharmaceutical Dawakhana Tibbiya College, Aligarh Muslim University, Aligarh since last many decades, described to be effective in liver diseases and is being prescribed by the Traditional Physicians, but has not been investigated scientifically so far, for its role in hepatic diseases.

MATERIALS AND METHODS

Collection and authentication of plant:

The ingredients of Kabideen were procured from local market of Aligarh then identified and authenticated by NISCAIR, New Delhi and the pharmacognosy section of the Department of Ilmul Advia, Aligarh Muslim University Aligarh.

Kabideen (syrup)

Kabideen is a polyherbal nopharmacopoeial formulation mainly prescribed for the management of liver disorders. It comprises of 21 ingredients, the details of the herbs are listed below in table No. 1.

Table No.1 Ingredients of kabideen

S.No.	Unani Name	Botanical Name	Parts used	Quantity
1	Biranjasif	*Achelli millefolium*	Top of Flowers	10gm
2	Barg-e-shahattara	*Fumaria officinalis*	Leaves	6gm
3	Barg-e-kasaundi	*cassia occidentalis*	Leaves	6gm
4	Tukhm-e kasni	*Cichorium intybus*	Seeds and root	10gm
5	Tukhm-e-Bathua	*Chenopodium alba*	Seeds	6gm
6	Tukhm-e-kasoos	*Cuscuta reflexa*	Seeds	6gm
7	Tukhm-e-khayarein	*C .sativa and c.melo*	Seeds	6gm
8	Mako	*Solanum nigrum*	Fruits	10gm
9	Rewand chini	*Rheum emodi*	Rhizomes	7gm
10	Sumbulut teeb	*Nordostachys jatamansi*	Rhizomes	6gm
11	Ood hindi	*Aquillaria agallocha*	Roots	6gm
12	Narmushk	*Ocrocorpus longifolius*	Buds	6gm
13	Satar Farsi	*Zataria multiflora*	Leaves	6gm
14	Ushba	*Smilax regelli*	Roots	6gm
15	Khulanjan	*Alpinia galanga*	Roots	6gm
16	Chiraita shireen	*Swertia chirata*	Leaves	3gm
17	Gul-surkh	*Rosa demascena*	Flowers	3gm
18	Gul nilofar	*Nymphae alba*	Flowers	6gm
19	Gul-e-tisoo	*Butea frondosa*	Flowers	10gm
20	Gul-e-ghafis	*Agrimonia eupatorium*	Flowers	10gm
21	Bekh-e-Kasni	*Cichorium intybus*	Roots	6gm

Dose: 25-50ml

Method of preparation

The decoction of the ingredient drugs was poured into a tin-coated vessel and added 2.5 parts of sugar, and then the vessel was kept on low fire and waited till the required consistency (Anonymous. (2006).

Experimental animals

Wistar albino rats of either sex weighing 100- 200g were used for the study. The animals were procured from Central Drug Research Institute Lukhnow. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30-70%. . All animals were fed on standard balanced diet and provided with water ad libitum.

CCl4 induced liver damage: Albino wistar rats of either sex (100-200g) were used. All the animals were divided into the five groups of 6 animals each and they received the treatment as follows.

Group I served as control and normal saline was given in the dose of 1ml/kg/p.o for 5 days.

Group II served as model for CCl_4 toxicity and CCl_4 was given as 1:1 in Olive oil in the dose of 1ml/kg/s.c on day 2 and 3.

Group III served as test group I and CCl_4 was given as 1:1 in olive oil in the dose of 1ml/kg/s.c on day 2 and 3. Simultaneously administered low dose of kabideen (3.5ml/kg/day,p.o) from 1- 5 days .

Group IV served as test group II and CCl_4 was given as 1:1 in olive oil in the dose of 1ml/kg/s.c on day 2 and 3. Simultaneously administered high dose of kabideen (7ml/kg/day,po) from 1- 5 days

Group V served as standard group and CCl_4 was given as 1:1 in olive oil in the dose of 1ml/k g/s.c on day 2 and 3. Simultaneously administered silymarin in the dose of 50mg/kg, p.o from 1- 5 days.

All animals were sacrificed on the 6th day and biochemical tests and histopathology were performed (Devipriya. D et al, 2007, T. Prakash et al 2008).

Biochemical estimation

After sacrificing the animals, the blood was collected and centrifuged at 7000 rpm for 15 minutes and stored at 4^0C. AST, ALT (Moss DW, Henderson AK, 1994), ALP (Kaplan A, Lavernel LS, 1983), Bilirubin (Malloy H.T and Evelyn K.A, 1937), Total protein (Kingsley G.R, 1939), Total cholesterol (Abell LL et al 1952) and lipid peroxidation (Ohkawa, H et al, 1979) were estimated in serum.

Histopathological examination: The livers of all animals were removed and preserved in 10% formalin solution for histopathalogical investigations (Luna LG, 1966).

Statistical Analysis: All the data expressed as Mean ± S.E.M and analyzed statistically using one way ANOVA tukey and compared with respective control group by graph pad instat. A value was of $P<0.05$ was considered significant.

RESULTS

Table 2, 3 and fig 1-7 represents that the administration of CCl_4 at a dose of 1 ml/kg (s.c.) caused a significant rise ($P<0.01$) in level of serum marker enzymes such as AST, ALT, ALP, bilirubin, cholesterol and MDA. Silymarin significantly ($P<0.01$) reduced these levels near to normal except total protein which was increased. A significant ($P<0.01$) decrease was observed in the AST, ALT, ALP, bilirubin, Cholesterol and MDA except total protein which was increased in the animals treated with low and high dose (3.5ml/kg, and 7 ml/kg) of kabideen that produced dose dependant activity whereas the high dose (7ml/kg) of kabideen showed nearly equal activity with the standard drug silymarin. Histopathological studies, (fig 8-12) revealed that ccl_4 produced extensive vascular degenerative changes and centrilobular necrosis in hepatocytes. Treatment with silymarin and high dose of kabideen reduced degenerative changes and centrilobular necrosis were found absent when compared with control group.

Table No 2. Effect of Test Drug Kabideen and Standard drug (silymarin) on CCl_4 induced toxicity

Group	S. ALT/SGPT (Units/ml) (Mean ± SE)	S.AST/SGOT (Units/ml) (Mean ± SE)	Serum Bilirubin (mg/dl) (Mean± SE)	S.Alk. Phosphatase IU/dl) (Mean± SE)	Total Protein (gm/100 ml)(Mean± SE)	Total cholesterol (mg/dl) (Mean ± SE)
Plain control	26.77± 0.741 Z*a*b*	51.65±0.94Z*a*b*	0.74±0.02 Z*a*b*	65.48±1.76 Z*a*b*	6.01±0.12 Z*a*b*	78.46±2.31 Z*a*b*
Ccl_4 toxicity 1ml/kg	89.02±2.98X*	94.09±1.69 X*	2.93±0.10X*	179.85±2.73 X*	4.29±0.11X*	162.23±4.53X*
Low dose of kbd 3.5ml/kg	50.47±1.51Y*	80.12±1.17 Y*	2.03±0.18Y*	164.31±2.25 Y*	4.49±0.03Y*	158.76±3.55Y*
High dose of kbd 7ml/kg	35.82±2.73Y*	65.27±1.36 Y*	1.25±0.03Y*	115.49± 4.47 Y*	5.09±0.15Y*	135.27±3.35Y*
Standard drug50mg/kg	32.32±1.96Y*	61.84±1.23 Y*	0.96±0.03Y*	92.95±5.69 Y*	5.42±0.20Y*	111.82±5.33Y*

n=6 x = against plain control y= against ccl4 (1ml/kg) z= against standard (silymarin 50mglkg)
*P< 0.001 a= against low dose kabideen (3.5ml/kg) b= against high dose kabideen (7ml/kg)

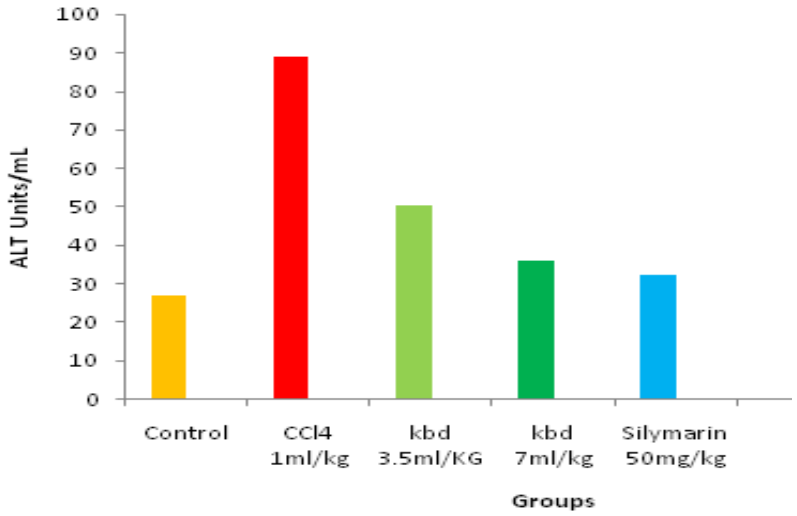

Fig1. Effect of Test drug on ALT in CCl_4 induced liver damage

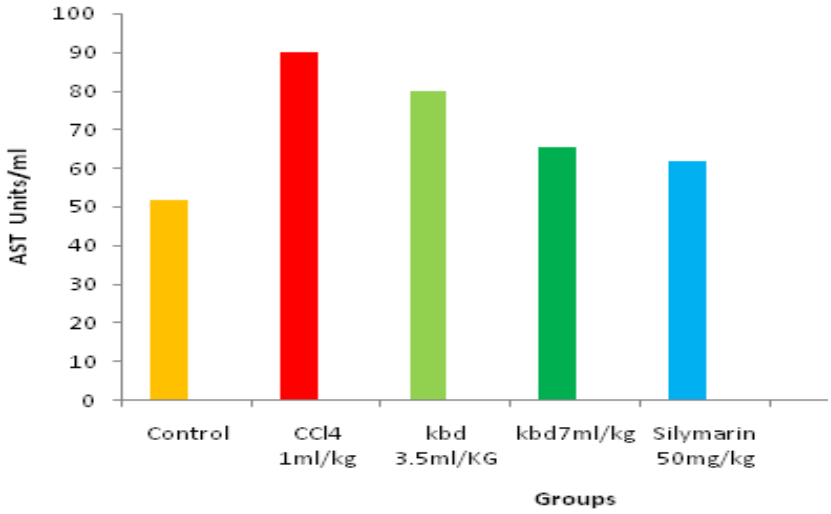

Fig2. Effect of Test drug on AST in CCl_4 induced liver damage

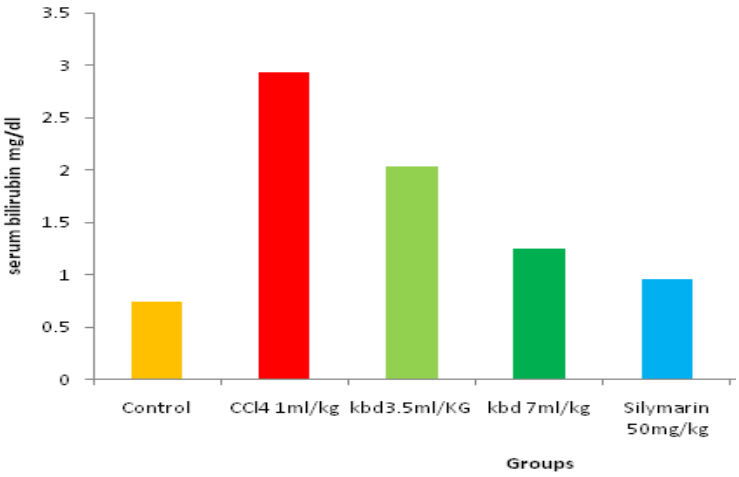

Fig3. Effect of Test drugs on Serum bilirubin in CCl_4 induced liver damage

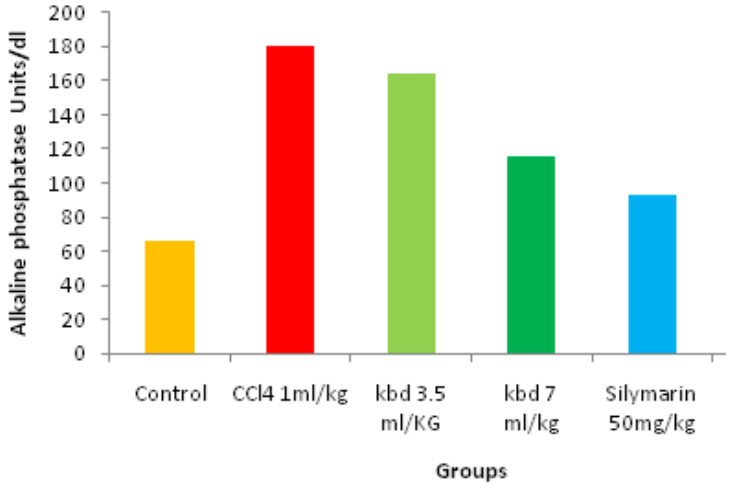

Fig4. Effect of Test drug on Alkaline Phosphatase in CCl_4 induced liver damage

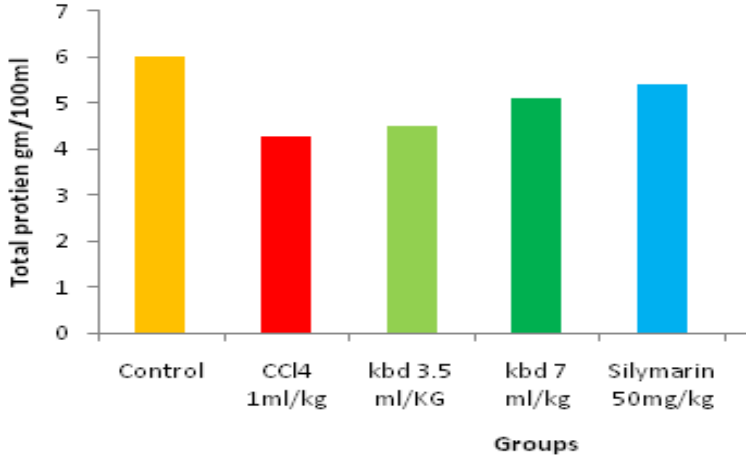

Fig 5. Effect of Test drug on total protein in CCl_4 induced liver damage

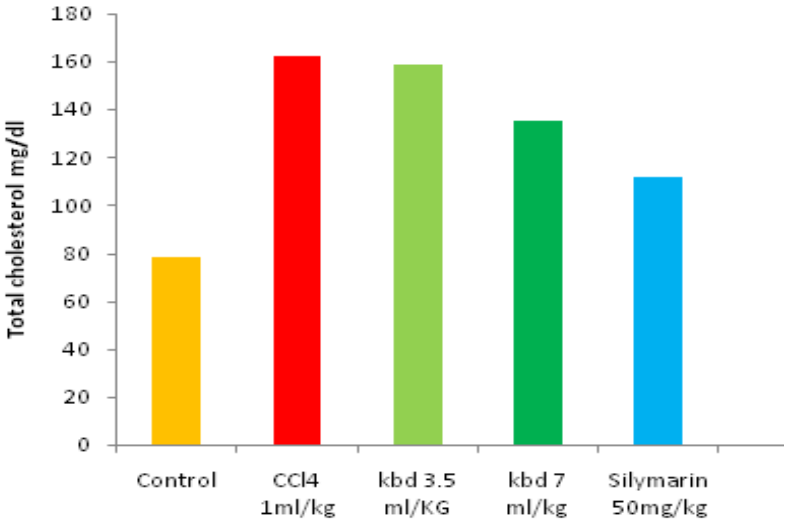

Fig 6. Effect f Test drug on Serum Cholesterol in CCl_4 induced liver damage

Table No 3. Effect of Test Drug kabideen and Silymarin (standard) on Lipid Peroxidation CCl_4 induced liver damage

Group	Lipid peroxidation (n mole of MDA/mg of protein) (Mean ± SE)
Plain control	1.99 ± 0.20Z*a*b*c*d*
Ccl4 toxicity 1ml/kg	8.78 ± 1.85X*
Low dose of S.P 3.5ml/kg + CCl_4 1ml/kg	7.65 ± 1.89Y*
High dose of S.P 7ml/kg + CCl_4 1ml/kg	2.38 ± 0.21Y*
Standard drug 50mg/kg + CCl_4 1ml/kg	2.32 ± 0.29Y*

n=6 x = against plain control y= against ccl4 (1ml/kg) z= against standard (silymarin 50mglkg)
*P< 0.001 a= against low dose kabideen (3.5ml/kg) b= against high dose kabideen (7ml/kg)

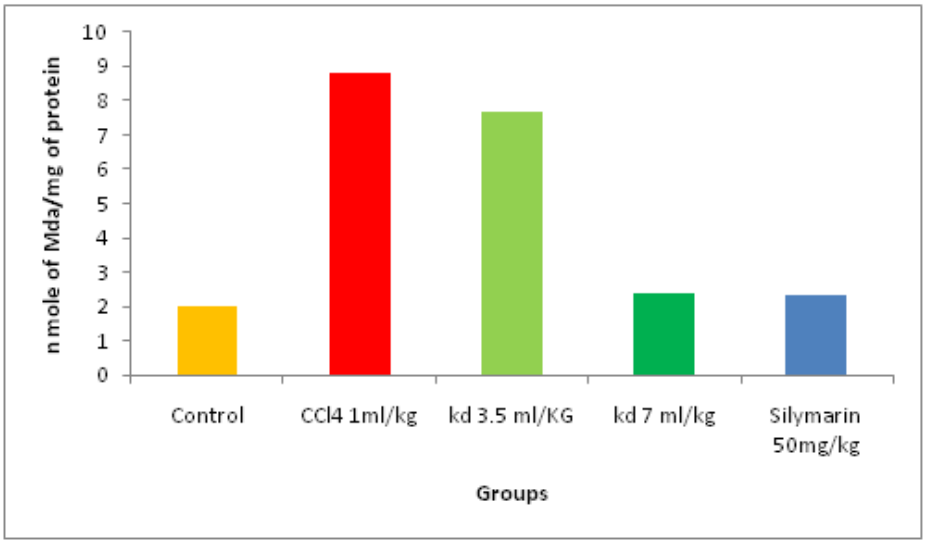

Fig 7. Effect of Test drug on n mole of MDA/mg of protein in CCl_4 induced liver damage

The histopathological findings of sacrificed liver of all groups are given below

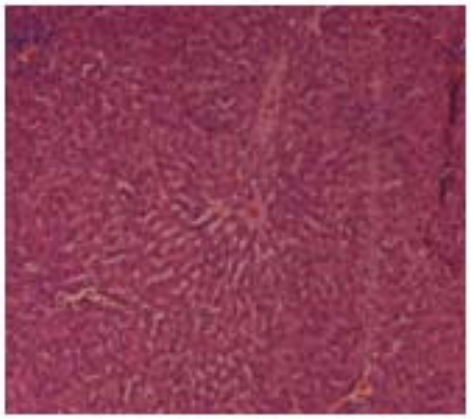

Fig-8 Normal liver Section showing prominent central vein & normal hepatocytes & sinusoids of liver

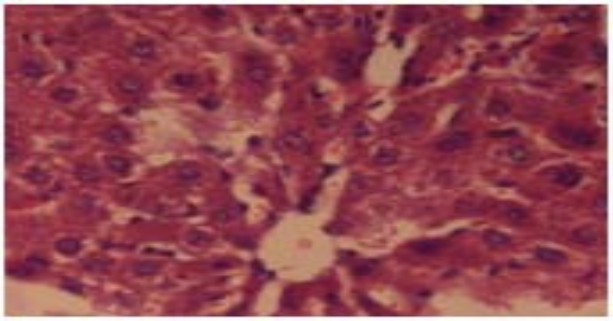

Fig-9 CCl_4 treated liver showing focal areas of liver cell necrosis and degeneration and fatty changes

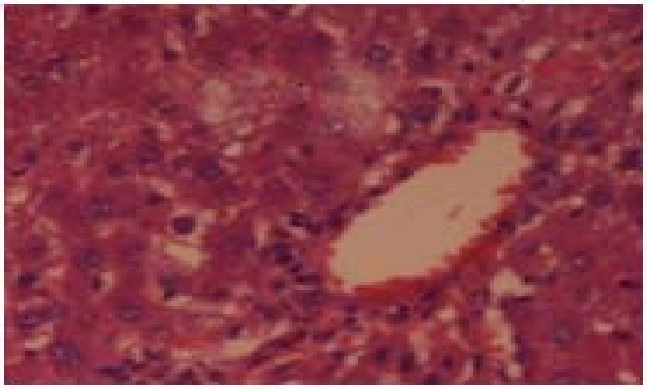

Fig-10 Kabideen low dose (3.5ml/kg) treated liver showing focal hepatocytes necrosis & inflammation

Fig-11 Kabideen high dose (3.5ml/kg) treated liver showing normal central vein and hepatocytes are seen with few areas of fatty changes.

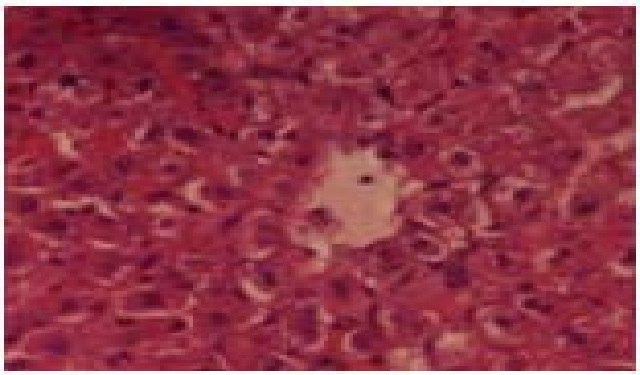

Fig-12 Silymarin treated liver showing normal hepatocytes and few areas of necrosis.

DISCUSSION

CCl4-induced hepatic injury is an experimental model widely used for the screening of hepatoprotective drugs. CCl_4 undergoes a biotransformation by hepatic microsomal cytochrome p450, to produce trichloromethyl free radicals. These hepatotoxic metabolites can react with protein and lipid in the membrane of cells or organelles leading to necrosis of hepatocytes. As a result of hepatic injury, the altered permeability of the membrane causes the enzymes from the cells to be released into the circulation. The magnitude of hepatic damage is usually assessed by measuring the level of released cytosolic transaminases including ALT and AST in the circulation. The rise in the serum levels of ALP, AST and ALT as observed in the present study could be attributed to the damaged structural integrity of the liver, because these are cytoplasmic in location and are released into circulation after cellular damage. Other researchers had reported that increased level of AST, ALT, ALP, bilirubin, cholesterol and decreased level of protein are due to CCl_4 hepatotoxicity. The increase in level of serum bilirubin is an index of degree of jaundice. It could be possibly be as a result of increased production, decreased uptake by liver, decreased conjugation, decreased secretion from liver. Table 2,3 and fig 1-8 represents that the administration of CCl_4 significantly elevated the levels of AST, ALT, ALP, cholesterol, lipid peroxidation and bilirubin, and decreased the total protein level due to damaged structural integrity of the liver because these are cytoplasm in location and are released into circulation after cellular damage. Kabideen prevented the CCl_4-induced perturbations in the activities of AST, ALT, ALP, cholesterol, lipid peroxidation, total protein and Bilirubin. The results in this study were confirmed by histopathological observations (fig 8-12). In contrast to the control group, CCl_4- intoxicant rat showed mild inflammatory cell infiltration and fatty changes, but kabideen administration for 5 days attenuated these histopathological changes.

CONCLUSION

The high dose of Kabideen (7ml/kg) exhibits significant hepatoprotective activity against hepatotoxicant like CCl_4 and this effect is nearly equal to the standard drug silymarin..It could be prescribed widely to normalize the diseased liver because of excessive use of chemical and industrial intoxicant. The present study proves kabideen as one of the potent polyherbal medications for liver ailment.

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