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Research Article

AN EXPERIMENTAL STUDY PERFORMED TO COMPARE THE HEPATOPROTECTIVE ACTIVITY OF *ALOE VERA* AND SILYMARIN IN CARBON TETRA CHLORIDE (CCL_4)-INDUCED HEPATOTOXICITY IN ALBINO RABBITS

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ABSTRACT

Objective: The aim of the study was to compare the hepatoprotective activity of *Aloe vera* and Silymarin in carbon tetrachloride (CCl_4)-induced hepatotoxicity in albino rabbits.

Methods: The study was conducted on 18 healthy albino rabbits of either sex weighing 1.5–2.0 kg, divided into three groups. Hepatotoxicity was induced in rabbits by administering CCl_4(0.05 mg/kg) intraperitoneally. Alcoholic extracts of leaves of *A. vera* and Silymarin were administered orally for 20 days from day 1 to day 20 in the doses of 100mg/kg/day with the help of a syringe in groups II and III respectively.

Results: Group I: There was an increase in the level of serum transaminase ($p<0.001$), serum alkaline phosphatase ($p<0.001$), serum bilirubin ($p<0.001$), and a decrease in serum albumin ($p<0.001$) due to hepatotoxic effect of CCl_4 when compared to day 0 of the same group. Group II: *A. vera* extract was found to reduce the level of aspartate transaminase ($p<0.0001$), alanine transaminase ($p<0.0001$), serum alkaline phosphatase ($p<0.0001$), serum bilirubin ($p<0.0001$), and increase in serum albumin ($p<0.0001$). Group III: Silymarin was found to reduce the level of aspartate transaminase ($p<0.0001$), alanine transaminase ($p<0.0001$), serum alkaline phosphatase ($p<0.0001$), serum bilirubin ($p<0.0001$), and increase in serum albumin ($p<0.0001$). The findings of Group II and Group III were found to be statistically highly significant when compared with Group I. On histopathology, Group II showed maximum reduction in fatty changes compared to Group III.

Conclusions: *A. vera* extract and Silymarin both showed a decline in hepatotoxic effects induced by CCl_4. Comparatively, *A. vera* exhibited higher protection in restoration of liver function and regeneration of liver cells than Silymarin.

Keywords: Hepatoprotective, *Aloe vera*, Silymarin, Albino rabbits, Carbon tetrachloride, Hepatotoxicity.

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INTRODUCTION

The largest internal organ in the body is the liver which contributes about 2% of total body weight and plays an essential role in the metabolism of foreign substances entering the body. These foreign substances are known as xenobiotics. The liver has a high regenerative capacity [1] and can often maintain function despite the significant disease. More than 1000 xenobiotic substances are potentially hepatotoxic [2]. Interaction of a series of complex processes involved in the uptake, biotransformation, and elimination of these potentially toxic compounds attributes to the ability of the chemical to produce liver damage often *in vivo*. There is a need to search for alternative drugs for the treatment of liver diseases to supplement the currently used conventional drugs that are often inadequate and of limited efficacy and safety.

There exists much interest in the possibility that antioxidants reduce the risk of degenerative diseases by inhibiting free radical-induced oxidative damage [3]. In the treatment of diseases such as hepatitis, jaundice, and loss of appetite, antioxidant properties present in the herb scan be used. For the hepatoprotective effect of indigenous drugs, the antioxidant property is claimed to be one of the mechanisms [4]. *Aloe vera* not only possesses hypoglycemic activity but is hypotensive and hepatoprotective. Silymarin is a hepatoprotective principle of the plant Silybum marinum. It has been described in the traditional system of medicine for its use in liver disease.

Therefore, the present research work was conducted to study the hepatoprotective effect of *A. vera* and Silymarin in carbon tetrachloride (CCl4) induced hepatotoxicity in experimental animals and the results are supported by histopathological evidence.

METHODS

Animals

The present study was conducted in the Department of Pharmacology and Therapeutics, G.S.V.M. Medical college, Kanpur after clearance from the Institutional Animal Ethical Committee for prevention of cruelty and supervision of experiments on the animal. A study was done on 18 healthy albino rabbits of either sex weighing 1.5–2.0 kg, divided into three groups. The animals were made available in the animal house of the Department of Pharmacology and Therapeutics, G.S.V.M. Medical College, Kanpur.

Rabbits also have a metabolism similar to human beings. Hepatotoxicity induced in rabbits by CCl_4 simulates the symptoms of drug-induced hepatitis in human beings without the development of concurrent infections. Hence, an experiment done on rabbits correlates well with human subjects.

A normal stock diet was given to all the animals for 7 days. The animals got acclimatized to the new environment during this time. All the animals were maintained under standard conditions (12 h light and dark cycle, at room temperature 25±3°C and 35–60% humidity) and were housed individually in a clean cage.

Drugs used

Alcoholic extract of leaves of *A. vera* and Silymarin were the drugs used in the study. Both drugs were administered orally for 20 days

from day 1 to day 20 with the help of a syringe. CCl_4 was administered intraperitoneally for 10 days from the 11th–20th day.

Preparation of extract

Leaves of *A. vera* were obtained from the Herbal garden of G.S.V.M. Medical College, Kanpur. Leaves gel of *A. vera* were obtained and the extract was prepared in 70% alcohol using the cold percolation method. After 7 days, extract of *A. vera* was collected. The alcohol-free extract was weighed and preserved at 4°C in a refrigerator.

Silymarin was obtained from the market as tablets. Silymarin tablets were crushed using mortar and pestle. The powder so obtained was dissolved in 1 ml of distilled water and was administered orally through a syringe followed by 1 ml of water.

CCl_4 was obtained from the market. Since CCl_4 is a hepatotoxic agent, it induces hepatitis in animals. Hepatitis causes anorexia and a decrease in body weight, Therefore, weight loss assessment was done in all the groups.

Each rabbit was given 60 g of diet that was provided between 11 A.M and 1.00 P.M daily. The amount consumed of the weighed diet given was calculated from the difference between the leftover amount of diet 24 h later. Water was given ad libitum.

Weight of the animals

Weight was recorded daily from the 1st day to the 20th day. Any decrease or increase in the weight of rabbits during drug administration was recorded.

Serum alanine transaminase, serum aspartate transaminase, serum alkaline phosphatase, serum bilirubin, and serum albumin estimation were done, blood samples were collected on day 0, day 11, and day 21.

Liver weight

Rabbits were sacrificed and the liver was taken out at the end of the study. It was weighed and preserved in 10% buffered formalin for histopathological study.

Procedure

Rabbits were divided into three groups. Each group consisted of six rabbits.

Group I: Animals were treated with hepatotoxic agent, that is, CCl_4 for 10 days in the dose of 0.05 ml/Kg/day intraperitoneally from day 1 to day 10 along with the normal feed. On the 11th day, blood samples were collected and rabbits were sacrificed.

Group II: Animals of this group were given an extract of leaves of *A. vera* (gel) 100 mg/Kg/day orally for 20 days along with normal feed, from 11th day onward CCl_4 0.05 mg/kg, i.p. was also given followed by herbal drug – *A. vera*.

Group III: Animals of this group were given Silymarin 100 mg/Kg/day orally for 20 days along with normal feed, from 11th day onward CCl_4 0.05mg/kg, i.p. was also given followed by drug Silymarin.

Blood samples were collected on day 0 before giving any drug to observe the control values of liver function tests (L.F.T), on day 11 to observe the *per se* effect of the herbal drug on L.F.T and on day 21 to observe the protective effect of the herbal drug on L.F.T. The values obtained were compared. Blood samples were drawn from the marginal vein of pinna using a 22 gauge needle after the ear hairs were shaved off. 3 ml blood was collected in the vial for the L.F.T.

Bodyweight was measured on daily basis. The animals of Group I were sacrificed on day 11 and the animals of Groups II and III were sacrificed on day 21. Ketamine was given to make animals unconscious. The abdomen was exposed and the liver was excised, weighed, and was preserved in 10% buffered formalin for histopathological study.

Assessment of liver injury

Assessment of liver injury was done by biochemical estimation and histopathological study of liver under a light microscope.

Biochemical estimation

Serum bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase, and serum albumin levels were estimated by Olympus autoanalyzer in the Department of Pathology, G.S.V.M. Medical College, Kanpur.

Histopathology

It was done to assess the extent of toxicity. After sacrificing the rabbit, the liver was taken out. It was weighed and preserved in 10% buffered formalin. Tissue was sectioned and slides were prepared. Hematoxylin and Eosin were used for staining purpose. Then, slides were examined under a light microscope and these slides were photographed.

Statistical calculations

Mean, standard deviation, and standard error of the mean were computed. Paired t-test and Student t-test were used for analyzing results and $p<0.05$ was considered as significant.

RESULTS

Effect on diet intake, body weight, and liver weight (Table 1)

In rabbits of Group I, who were administered CCl_4 (0.05 mg/kg/day, intraperitoneally) along with normal feed, the diet intake was found to be 39.13±0.77 g/day. The decrease in food intake has led to a decrease in body weight. The mean decrease in body weight in Group I was considerably more when compared to Group II and Group III. The mean weight of the liver recorded was 28.38±0.18 g.

In rabbits of Group II (who received *A. vera* extract), the average diet intake was 60.34±0.52 g/day during the first 10 days. On adding CCl_4 from day 11 onwards, the average diet intake was 56.15±0.34 g/day (in the past 10 days), showed a decrease by 6.9% and increase by 28.4 % when compared to average diet intake during the first 10 days of the same group and with the Group I, respectively. The mean weight of the liver was measured to be 52.36±0.35.

In rabbits of Group III (received Silymarin), the average diet intake was 60.44±0.54 g/day during the first 10 days. On adding CCl_4 from day 11 onward, the average diet intake was 49.17±0.13 g/day (in the past 10 days), which showed a decrease by 18.3% and increase by 16.6 % when compared to average diet intake during the first 10 days of the same group and with the Group I, respectively. The mean weight of the liver recorded was 38.47±0.22.

LFT (Tables 2-6)

In Group I, a highly significant ($p<0.001$) increase in the levels of serum transaminases, serum alkaline phosphatase, serum bilirubin, and a significant decrease in serum albumin with $p<0.001$ was observed compared to day 0 of the same group (self-control).

Administration of *A. vera* extract and Silymarin into rabbits, fed on a normal diet, did not alter the level of serum transaminase ($p>0.10$), serum alkaline phosphatase ($p>0.10$), serum bilirubin, and serum albumin ($p>0.10$) when compared to day 0 of the same group.

In Group II, *A. vera* extract was found to reduce the levels of serum transaminase (Tables 2 and 3), serum alkaline phosphatase (Table 4), serum bilirubin (Table 5), and increase in serum albumin (Table 6). The results were statistically highly significant ($p<0.001$) when compared with the 11th day of rabbits receiving CCl_4 alone.

In Group III, Silymarin was found to reduce the levels of serum transaminase (Tables 2 and 3), serum alkaline phosphatase (Table 4), and serum bilirubin (Table 5). The results were found to be statistically highly significant ($p<0.001$) when compared with the 11th day of rabbits receiving CCl_4alone. Further, Silymarin significantly increased the

Table 1: Average diet intake per day, mean liver weight (g) of rabbits, and mean body weight (g) of different groups

Group	Average Diet Intake (g/kg) 1st–10th day	Average Diet Intake (g/kg) 11th–20th day	Mean Liver Weight (g)	Average Body weight (in kg) 1st–10th day	Average Body weight (in kg) 11th–20thday
I (CCl_4)	39.13±0.77	-$	28.38±0.18	1.04±0.11	-$
II (*Aloe vera* extract)	60.34±0.52	56.15±0.34	52.36±0.35	1.59±0.34	1.52±0.38
III (Silymarin)	60.44±0.54	49.17±0.13	38.47±0.22	1.60±0.40	1.51±0.42

All values are in MEAN±SE, $ Group I rabbits were sacrificed on 11thday

Table 2: Mean aspartate transaminase (AST/SGOT) in IU/L of rabbits in different groups

GROUP (n=6)	Mean SGOT±SE (IU/L) At 0-day	Mean SGOT±SE (IU/L) At 11th day	Mean SGOT±SE (IU/L) At 21st day
I (CCl_4)	30.67±0.88	87.17±1.58*	-$
II (*Aloe vera* extract)	29.50±1.18	30.00±1.37**	54.17±0.79*,#
III (Silymarin)	29.00±1.53	29.67±1.69**	57.33±1.45*,#

*p<0.001, **p>0.10, *,** values are compared with 0 days of same group (self-control), # p<0.0001,# compared with 11th day of Group I (CCl_4). $ Group I rabbits were sacrificed on 11th day

Table 3: Mean alanine transaminase (ALT/SGPT) in IU/L of rabbits in different groups

Group (n=6)	Mean SGPT±SE (IU/L) At 0 day	Mean SGPT±SE (IU/L) At 11th day	Mean SGPT±SE (IU/L) At 21st day
I (CCl_4)	30.33±1.49	132.67±3.77*	-$
II (*Aloe vera* extract)	29.17±1.82	29.33±1.38**	53.33±1.94*,#
III (Silymarin)	29.67±1.71	29.50±1.17**	71.17±0.87*,#

*p<0.001, **p>0.10,$ Group I rabbits were sacrificed on 11th day, *,** values are compared with 0-day of same group (self-control), #p<0.0001,# compared with 11th day of Group I (CCl_4)

Table 4: Mean alkaline phosphatase (ALP) in IU/L of rabbits in different groups

Group (n=6)	Mean ALP±SE (IU/L) At 0-day	Mean ALP±SE (IU/L) At 11th day	Mean ALP±SE (IU/L) At 21st day
I (CCl_4)	36.50±2.53	121.83±3.07*	-$
II (*Aloe vera* extract)	39.33±2.77	39.00±3.35**	48.67±1.94***,#
III (Silymarin)	33.17±2.41	36.00±2.37**	63.17±1.38*,#

*p<0.001, **p>0.10,***p<0.05, $ Group I rabbits were sacrificed on 11th day, *,**,*** values are compared with 0 day of same group (self-control), #p<0.0001,# compared with 11th day of Group I (CCl_4)

Table 5: Mean serum bilirubin (mg/dl) of rabbits in different groups

Group (n=6)	Mean Serum Bilirubin (mg/dl)±SE At 0 day	Mean Serum Bilirubin (mg/dl)±SE At 11th day	Mean Serum Bilirubin (mg/dl)±SE At 21st day
I (CCl_4)	0.33±0.04	1.07±0.07*	- $
II (*Aloe vera* extract)	0.37±0.03	0.38±0.03**	0.50±0.03***,#
III (Silymarin)	0.35±0.04	0.37±0.04**	0.55±0.04*,#

*p<0.001, **p>0.10,*** p<0.01, $ Group I rabbits were sacrificed on 11th day, *,**,*** values are compared with 0 day of same group (self-control), #p<0.0001,# compared with 11th day of Group I (CCl_4)

Table 6: Mean serum albumin (g/dl) of rabbits in different groups

Group (n=6)	Mean Serum Albumin (g/dl)±SE At 0-day	Mean Serum Albumin (g/dl)±SE At 11th day	Mean Serum Albumin (g/dl)±SE At 21st day
I (CCl_4)	4.00±0.10	2.33±0.07*	-$
II (*Aloe vera* extract)	3.93±0.15	4.02±0.17**	3.67±0.12***,#
III (Silymarin)	4.17±0.10	4.20±0.07**	4.17±0.09**,#

*p<0.001, **p>0.10,*** p<0.02. $ Group I rabbits were sacrificed on 11th day, *,**,*** values are compared with 0 day of same group (self-control), #p<0.0001,# compared with 11th day of Group I (CCl_4)

serum albumin level comparable to day 0 of the same group, which was found to decrease with CCl_4 administration.

Histopathological assessment

Rabbits administered CCl_4

Grade III fatty changes and hydropic degeneration was present in 75% of rabbits and Grade II fatty changes were present in 25% of rabbits. Centrilobular (perivenular) and periportal inflammation were found in 75% and 25% of rabbits, respectively, chiefly infiltrated with monocytes. Grade II inflammation was present in all rabbits. Grade II necrosis and loss of cord pattern were found in all rabbits (Fig. 1).

Rabbits administered *A. vera* extract and CCl_4

When rabbits on CCl_4 were compared with those receiving *A. vera* extract and CCl_4, there was maximal protection of hepatic lobules from the damage induced by CCl_4. Grade I fatty changes in 30% of rabbits were present. Portal inflammation of Grade I, infiltrated by monocytes, was present in 30% of rabbits. There was no area of necrosis. The cord pattern was maintained (Fig. 2).

Rabbits administered Silymarin and CCl_4

When rabbits on CCl_4 were compared with those receiving Silymarin and CCl_4, there was some protection of hepatic lobules from the damage induced by CCl_4. Grade I fatty changes in 70% of rabbits were present. Foamy hepatocytes and fine vacuole were present. Portal inflammation of Grade I, infiltrated by lymphocytes, was present in 70% of rabbits, and necrosis of Grade I in the centrilobular zone was found in 20% of rabbits. The cord pattern was maintained (Fig. 3).

The results of this study suggest that administration of *A. vera* extract and Silymarin, individually to rabbits followed by CCl_4 administration from 11th day to 20th day showed a decline in hepatotoxic effects induced by CCl_4, which was evidenced by a marked decrease in serum aspartate transaminase and alanine transaminase levels relative to the group treated with CCl_4 alone. Furthermore, *A. vera* extract showed higher protection of the liver than Silymarin.

DISCUSSION

Environmental pollution, food additives, cosmetics products, agrochemicals, processed food, and drugs are the sources that include most xenobiotics to which humans are exposed. In general, in the

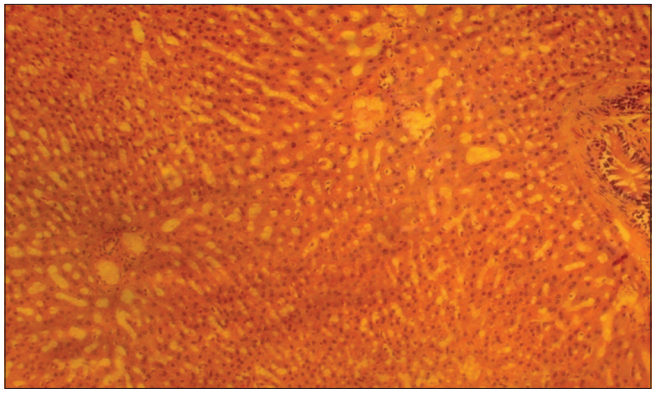

Fig. 1: A section of rabbit liver treated with CCl_4 alone showing marked fatty changes and Grade II inflammatory changes in 100% area and loss of cord pattern

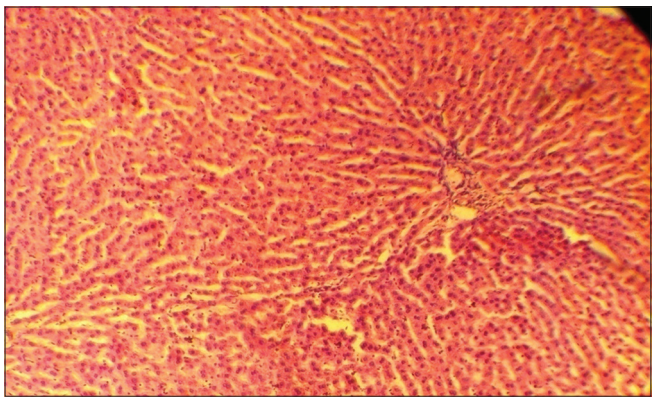

Fig. 2: A section of rabbit liver treated with *Aloe vera* extract and CCl_4 showing maximum protection from damage induced by CCl_4. (Grade I fatty changes in 30% and Grade I Portal inflammation in 30% area)

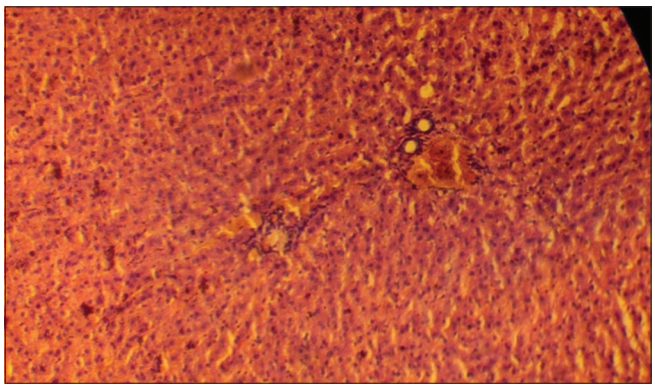

Fig. 3: A section of rabbit liver treated with Silymarin and CCl_4 showing protection from damage induced by CCl_4.(Grade I fatty changes in 70% and Grade I portal inflammation in 70% area)

absence of metabolism, these chemicals would not be eliminated from the body efficiently, and thus would accumulate in the body resulting in toxicity. Hepatic injury is a common sequel of exposure to toxic agents.

One of the most commonly used hepatotoxins in the experimental study of liver diseases is CCl_4 [5]. Plant-derived natural products such as flavonoids, terpenoids, and steroids, due to their diverse pharmacological properties including hepatoprotective and antioxidant activity have received considerable attention in recent years [6,7]. Therefore, this study was carried out to evaluate the hepatoprotective effect of *A. vera* extract and Silymarin which are plant derivatives.

In our study, we found an increase in the average intake of diet by 28.4% in Group II and 16.6% in Group III which received *A. vera* extract and Silymarin, respectively, compared to Group I that received CCl_4 alone. The mean weight of the liver recorded was found to be more in Group II (52.36±0.35) and Group III (38.47±0.22) compared to Group I (28.38±0.18 g). Our finding suggests that *A. vera* extract and Silymarin were able to arrest the decrease in the weight of the liver when compared to CCl_4 administered group.

The physiological state of the liver is reflected by the serum level of marker enzymes: Alanine transaminase, aspartate transaminase, and alkaline phosphatase. These enzymes change according to the distortion of the liver that results from cellular injury of the organ caused by toxic metabolites and diseases. Serum and plasma enzyme levels have been used as a marker for monitoring chemically induced tissue damages [8,9]. The increase in serum transaminases and alkaline phosphatase indicates the cellular leakage and loss of functional integrity of cell membrane where the increase in levels of serum bilirubin reflects the depth of jaundice. In acute hepatotoxicity, liver enzymes are usually raised but tend to decrease with prolonged intoxication due to damage to liver cells.

In our study, we found a highly significant ($p<0.001$) reduction in the levels of serum transaminases, serum alkaline phosphatase, serum bilirubin, and increase in serum albumin levels in Group II and Group III compared to Group I that showed highly significant ($p<0.001$) increase in the levels of serum transaminases, serum alkaline phosphatase, serum bilirubin, and decrease in serum albumin levels. Our finding suggests that *A. vera* extract and Silymarin were able to produce a decline in hepatotoxic effects induced by CCl_4 as evidenced by serum enzyme levels.

The toxicity to the liver of mammals is largely due to the active metabolite, trichloromethyl radical of CCl_4 [10]. This radical binds to tissue macromolecules and thus induces peroxidative degradation of membrane lipids of the endoplasmic reticulum (ER), which are rich in polyunsaturated fatty acids. It has been postulated that such development would ultimately lead to the formation of lipid peroxides [11]. The increase in the plasma enzyme levels of CCl_4 treated rabbits suggests that the toxicant was able to reach the liver and induce detectable damage.

In our study, the histopathological assessment showed portal inflammation of Grade I present in 30% of rabbits in Group II and 70% of rabbits in Group III compared to Group I where Grade II inflammation and loss of cord pattern were found in all rabbits. Our finding suggests that *A. vera* extract and Silymarin were able to protect hepatic lobules from the damage induced by CCl_4, where *A. vera* exhibited a higher hepatoprotective effect than Silymarin.

A. vera is a drought-resistant tropical plant from the Liliaceae family that exhibits strong antioxidant properties [12] and has a broad range of applications in traditional medications. It contains a large number of bioactive compounds such as terpenoids, lectins, flavonoids, fatty acids, tannins, sterols, anthraquinones, mono and polysaccharides, enzymes, salicylic acid, minerals, and vitamins [12]. *A. vera* reduced the formation of lipid peroxidation was determined in a study of the effects of *A. vera* gel against oxidative stress-induced liver damage [13]. In some other studies, it was found that iNOS (inducible nitric oxide synthase involved in acute hepatotoxicity) expression levels are decreased in the *A. vera* -treated rats in the liver injury induced by CCl_4 [14]. The hepatoprotective potential of *A. vera* was also confirmed in another study that was done to evaluate the hepatoprotective activity of *A. vera* in acute viral hepatitis [15].

Silybum marianum L. is an ancient medicinal plant that belongs to Asteraceae/Compositae family and has been used in the treatment of different liver diseases [16]. Three potent bioflavonoids: Silybin, Silydianin, and Silychristin are the active substances collectively

known as Silymarin. Antioxidant, free radical scavenging property, and stimulation of protein synthesis are the most important hepatoprotective mechanisms of Silymarin [16]. Silymarin has led to complete normalization of elevated transaminases levels when compared with various polyherbal formulations in CCl-induced hepatotoxicity in rats [16]. Previously done studies have shown that Silymarin was effective in preventing poisoning by several hepatotoxic substances, including CCl_4 [17]. The findings of these different studies done in the past and recent strengthen our study results that suggest the hepatoprotective role of *A. vera* and Silymarin. Furthermore, when compared to Silymarin, *A. vera* showed higher hepatoprotection.

CONCLUSIONS

From the above discussion, it is clear that CCl_4 administration produces hepatic injury as is evident both by the changes in the biochemical parameters and histopathological changes reported in the present study. There is evidence of the varying degree of oxidative stress leading to hepatocellular damage. We observed that extracts of leaves of *A. vera* and Silymarin protect the liver against CCl_4-induced hepatotoxicity.

A. vera extract had better efficacy in reducing enzyme levels of alanine transaminase, aspartate transaminase, alkaline phosphatase, and S. bilirubin than Silymarin. Furthermore, *A. vera* extract has shown higher protection in restoration of liver function and regeneration of liver cells than Silymarin as observed on histopathology. Silymarin had better efficacy in increasing S. albumin than *A. vera*.

Therefore, we concluded that herbal preparations such as *A. vera* and Silymarin showed hepatoprotective efficacy but as this study was done on a small scale and for a short duration, further research is needed to explore more about the active principle and mechanism of action responsible for their hepatoprotective activity.

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