Hepatoprotective Effect of Aloe vera Against Carbon Tetrachloride Induced Hepatotoxic Effects in Experimental Animal Models

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ABSTRACT

Background: Aloe vera, is a natural hepatoprotective agent used alone or as an ingredient of number of medicinal preparations in Indian traditional medicine (Ayurveda) and in folk medicine across the globe for different liver ailments. However, considerable number of them lacks scientific proof for their claims. Hence, Aloe vera was evaluated for its protective effect against carbon tetrachloride (CCL) induced liver toxicity in wistar albino mice and rabbits.

Materials and methods: Two species of the animals (rabbit and mice) were used to evaluate Aloe vera for its effects on histopathology, biochemistry and mortality induced by carbon tetrachloride in experimental setup. The animals were divided into main three groups (GI, GII and GIII) of eighteen animals each. Each group was further divided into three subgroups of six animals each. Depending upon the treatment protocol, subgroup ‘a’ received linseed oil as control. Subgroup ‘b’ received CCL, while as subgroup ‘c’ received extract of Aloe vera prior to CCL administration.

Results: Animals pretreated with Aloe vera and subsequently treated with CCL showed significant reduction in histopathological changes (p<0.05), biochemical changes (p<0.05) and mortality rate (p<0.05) in comparison to the animals treated with CCL alone. Conclusion: This study reveals the hepatoprotective effect of Aloe vera against CCL induced liver toxicity.

Key words: Aloe vera, hepatoprotection, carbon tetrachloride, animal models


INTRODUCTION

Liver plays a significant role not only in metabolism and detoxification of exogenous toxins and therapeutic agents, but also in the bio-regulation of fats, carbohydrates, amino acids and proteins (Alkasoumi et al., 2008). A number of pharmacological and chemical agents act as hepatoprotectins and produce a variety of liver ailments (Ram, 2001). Though tremendous strides have been made in the last four decades in modern medicine, yet there is no drug that protects liver from damage or helps in the regeneration of hepatic cells. Corticosteroids are used empirically to prevent further fibrosis and check antigen antibody reaction but no specific action on the liver has been demonstrated.

Numberless drugs of indigenous origin were investigated by variety of animal experiments to see if they could help in the protection of regeneration of the hepatic parenchyma. Aloe vera is one such preparation used alone as a sole agent or one of the ingredients in many preparations in Indian traditional medicine (Ayurveda) and in other corners of the world as hepatic stimulant and in liver enlargement (Mossa et al., 1987; Vazquez et al., 1996; Reynolds and Dweck, 1999; Can et al., 2004; Boudreau and Beland, 2006; Miliadi and Damak, 2008). Moreover, Aloe vera is used and has been evaluated for its potential role in many other clinical conditions (Langmead et al., 2004; Vinson et al., 2005; Essau and Rauwald, 2006; Hamman, 2008; Ramachandra and Rao, 2008). Though numerous herbal extracts are used for liver problems, however, considerable number of them lacks scientific proof for their claims. Hence, it was found worthwhile to evaluate the hepatoprotective effect of Aloe vera against carbon tetrachloride induced hepatotoxicity in experimental animal models.

MATERIALS AND METHODS

Animals: Healthy Wistar albino mice of either sex weighing between 20-30 g and wistar albino rabbits weighing between 1-2 kg were used for histopathological, survival and biochemical study to evaluate the hepatoprotective effect of Aloe vera. The animals were housed within the departmental animal house and the room temperature was maintained at 27°C. They were provided with Purina chow and free access to...
water ad libitum (Abdel-Kader and Alqasoumi, 2008). The protocol was approved and carried out after the permission of Institutional Animal Ethics Committee (IAEC).

**Investigational drugs and dosage preparations:** The appropriate body weight adjusted doses of extract of Aloe vera as extrapolated from doses used in similar studies conducted previously to be 0.2 mL kg⁻¹ for mice and 2.5 mL kg⁻¹ for rabbit were used (Alqasoumi et al., 2008). The formulations were fed to the animals through gastric tube (18 to 22 mm) for rabbit and 2-3 mm polythene tubing sleeved on an 18-20 gauge blunted hypodermic needle for mice (Ahmed et al., 2010). The extract of Aloe vera (L.) Burm. f. (Asphodeleaceae) was obtained from E. Merck.

**Experimental protocol:** Animals (n = 54) consisting of mice (n = 36) and rabbits (n = 18) were allocated to three main groups of 18 animals each. Depending upon the treatment design, animals were further subdivided into three groups of six animals each for different experimental tests. The linsseed oil alone was used as control in the subgroups I, II, and III. Carbon tetrachloride (CCl₄) mixed with equal volume of linsseed oil was used as hepatotoxic agent in the subgroups I, II, and III. While as the extract of Aloe vera was used in subgroups I, II, and III prior to CCl₄ administration. Mice were used for histopathology and survival tests. However, rabbits were used for biochemical tests.

**Histoprotective test:** Three groups (GI, GIi, and GI) of six mice each were used. GI received linsseed oil as control. GI received 2.5 mL kg⁻¹ of 50% CCl₄ solution in linsseed oil orally. However, GI, received 0.2 mL kg⁻¹ of extracts of Aloe vera daily for seven days and on the eighth day 2.5 mL kg⁻¹ of 50% CCl₄ solution was given orally. After 48 h following CCl₄ administration the animals were sacrificed using ether anesthesia. The liver was immediately removed and a small piece was fixed in 10% formalin and subsequently stained with hematoxilin and eosin for histopathological assessment.

**Histopathology examination:** Under aseptic procedure the liver of all the treated animals in group GI were removed and a piece of tissue was fixed in 10% formalin for histological assessment. Following the procedure the sections were ultimately placed onto the clean slides that were drained vertically for several minutes before placing them onto a warming table at 37-40°C (Prophet et al., 1994). The slides were dehydrated and cleared and finally mounted with resinous medium. While assessing the liver biopsies the control group (GI) showed normal histological appearance. The architecture of liver was maintained in CCl₄ treated group (GI) of mice. Hepatocytes showed cloudy and fatty degeneration, central veins and sinusoids were dilated with infiltration of polymorphonuclear cells and few mononuclear cells. Necrosis of hepatocytes were seen which was mostly of centrilobular type. However, in group (GI) of mice which were pretreated with Aloe vera and subsequently with CCl₄, the architecture of liver was maintained. Hepatocytes showed little cloudy and fatty degeneration, central veins and sinusoids were not dilated. Most importantly areas of necrosis was not seen.

**Survival test (Mortality scores):** Three groups (GIIL, GIIL, and GIIL) of six mice each were used. GI received linsseed oil as control. GI received 2.5 mL kg⁻¹ of 50% CCl₄ solution in linsseed oil. GI received 0.2 mL kg⁻¹ of extracts of Aloe vera daily for seven days and on the eighth day 2.5 mL kg⁻¹ of 50% CCl₄ solution was given orally. All the animals in each group were observed for one week and the mortality rate of animals in each group was recorded and compared among the treatment groups.

**Biochemical test:** Three groups (GIIL, GIIL, and GIIL) of six rabbits each were used. GI received linsseed oil as control. GI received 2.5 mL kg⁻¹ of 50% CCl₄ solution in linsseed oil. GI received 2.5 mL kg⁻¹ of extracts of Aloe vera daily for seven days and on the eighth day 2.5 mL kg⁻¹ of 50% CCl₄ solution in linsseed oil was given orally. After 24 h of CCl₄ administration, 2 mL of blood sample was collected from marginal ear vein of all the animals for biochemical parameter (SGOT, SOPT and serum protein) estimation (Boon et al., 2006). The enzyme activities were estimated by employing diagnostic strips (Reflotron®, ROCHE) and were read on a Reflotron® Plus instrument (ROCHE).

**Statistical analysis:** The statistical analysis was done by using Chi-square test for analysis of the results of histopathological and survival tests. However, Student’s ‘t’ test was employed for analysis of the results of biochemical test. A probability value of less than 0.05 (p<0.05) was considered to be statistically significant. The results are expressed as Mean±SEM.

**RESULTS AND DISCUSSION**

While assessing the results of this study for evaluation of hepatoprotective action of Aloe vera, a natural remedy used in various medicinal preparations in Indian traditional medicine (Ayurveda) and folk medicine in other corners across the globe (Grindle and Reynolds, 1986; Davis et al., 1989; Rajasekaran et al., 2005; Madan et al., 2008). The present study was designed to test the Aloe vera for its role as hepatoprotective agent against carbon tetrachloride induced hepatic injury and histological changes. Two species of the animals (rabbit and mice) were used to evaluate Aloe vera for its effects on histopathology, biochemistry and mortality induced by carbon tetrachloride in experimental setup.
Fig. 1: CCl₄ induced histopathological changes and protective effect of Aloe vera on mice. Each point represents percentage of histological changes (n = 6) *p<0.05 vs. CCl₄ group (Chi-square test). CCl₄: Carbon tetrachloride, n: No. of animals

Fig. 2: CCl₄ induced mortality and protective effects of Aloe vera on mice. Each point represents percentage of mortality (n = 6) *p<0.05 vs. CCl₄ group (Chi-square test). CCl₄: Carbon tetrachloride, n: No. of animals

Mice were used to study histopathological changes, mortality scores and rabbits for biochemical changes induced by carbon tetrachloride.

In histopathological examination, the liver biopsies of control group showed normal histological appearance. The animals treated with CCl₄ showed centrilobular hepatocyte necrosis, extensive fatty degeneration and infiltration by polymorphonuclear cells. Exposure to CCl₄ leads to histopathological changes from the normal histological appearance (Alqasoumi et al., 2008). However, the degree of histopathological changes has significantly reduced (p<0.05) in animals pretreated with Aloe vera and subsequently treated with CCl₄ in comparison to the animals treated with CCl₄ alone. The architecture of liver was maintained, hepatocytes showed little cloudy and fatty degeneration. Moreover areas of necrosis were not seen, central veins and sinusoids were not dilated (Fig. 1).

Assessing the mortality score, no mortality was recorded in control group. 100% mortality occurred in CCl₄ treated group within 48 h after oral administration of CCl₄. However, this mortality rate significantly reduced (p<0.05) in a group pretreated with Aloe vera and subsequently treated with CCl₄ (Fig. 2) as only 33% mortality was recorded in this group.
While assessing the biochemical parameters, an increase in SGOT, SGPT and serum protein was recorded in a group treated with CCl₄ as compared to control and Aloe vera treated groups. Treatment of animals with the hepatotoxic agent CCl₄ resulted in significant increase of transaminases (SGOT and SGPT) and alkaline phosphate levels (ALP) due to hepatocyte damage (Zafar and Ali, 1998). Severe jaundice was reflected by increased level of serum bilirubin (Lin et al., 1997). However, this increase of transaminases (SGOT and SGPT) and serum protein was significantly inhibited (p<0.05) in a group pretreated with Aloe vera and subsequently treated with CCl₄ (Fig. 3). Our results are in accordance with the study stating animals (rats) treated with Aloe vera extract exhibited a significant reduction only in SGOT level at the lower dose. Where as the higher dose significantly lowered the levels of SGOT, SGPT, ALP and bilirubin, respectively, indicating a good level of protection against the toxicity of CCl₄ (Algasoumi et al., 2008).

CONCLUSION

This study reveals hepatoprotective effect of Aloe vera in terms of its hepatoprotective effect, no significant increase in biochemical parameters and significant reduction in mortality rate against CCl₄ challenge as hepatotoxic agent.

REFERENCES


