EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF AQUEOUS EXTRACT OF ALOE VERA

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Abstract

The Aim of this study was to investigate the hepatoprotective effect of aqueous extract of Aloe vera against carbon tetrachloride induced hepatotoxicity in rats. 36 Albino wistar rats (150-200g) were taken & divided into six groups. I group was given normal saline (2ml/100g orally), II group received 1 ml/kg of carbon tetrachloride intraperitoneally (i.p.), III group was given Liv.52 (2 ml/kg) orally for ten days followed by carbon tetrachloride i.p. on 10th day. IV, V & VI groups received Aloe vera extract (2ml/100g/day) for 10, 20 & 30 days respectively followed by carbon tetrachloride i.p on 10th, 20th & 30th days. Animals of all the groups were fasted for 24 hours. Then, the rats were anaesthetized with ketamine (75 mg/kg) and diazepam (10 mg/kg). Blood was collected & then animals were sacrificed to take out the liver for histopathologica examination. The sera were used to estimate alanine aminotransferase, alkaline phosphatase & total bilirubin and liver were subjected to histopathologica examination. Alkaline phosphatase, alanine transaminase & total bilirubin level in normal saline treated group were in normal range, while these were significantly increased in carbon tetrachloride treated group. Aloe vera extract displayed significantly low values of alkaline phosphatase, alanine aminotransferase & bilirubin in group III, IV & V. This hepatoprotection by Aloe vera was also reflected in histology. It can be concluded that Aloe vera extract is a potent hepatoprotective agent.
INTRODUCTION:

Liver is the largest organs in human body. It is the chief site for intense metabolism and excretion. So it is continuously and variedly exposed to environmental toxins, and abused by poor drug habits and alcohol, which can eventually lead to various liver disorders.

Till date available modern drugs have not been able to come up with a satisfactory answer for liver disorders\(^1\)\(^2\) (Karan et al., 1999; Chaterrjee et al., 2000). In the absence of reliable liver protective drugs in allopathic system of medicine, herbs are claimed to have important role in the management of various liver disorders. Liv.52 is such a kind of herbal formulation of The Himalaya Drug Company India. It is a mixture of extracts of 7 herbs viz Capparis spinosa, Cichorium intybus, Solanum nigrum, Terminalia arjuna, Cassia occidentalis, Achillea millefolium, Tamarix gallica and used for the treatment of various liver diseases. It has more than 250 research articles published in its favor including experimental as well as clinical studies in various national and international journals.\(^3\)

Aloe vera is a plant which belongs to asphodelaceae family and has been shown to be hepatoprotective by the Ayurvedic system of medicine in India and North America\(^4\) (http://www.karinya.com/liver.htm) as well as its uses in other diseases like in heart burn, irritable bowel syndrome, ulcerative colitis, diabetes mellitus, hyperlipidaemia and as an immunostimulant.

However, the Literature reviews indicate that the hepatoprotective activity of Aloe vera has not been scientifically evaluated so far. Therefore, the present study is intended to evaluate hepatoprotective activity of aqueous extract of Aloe vera in experimentally induced hepatotoxicity in albino rats.

MATERIALS AND METHODS

Preparation of plant extract

The Aloe vera plant was collected from the local market of Meerut, UP (India) in August 2009. The plant was authenticated by the
Botany Department of CCS University, Meerut, UP (India). The leaves were washed with clean water, cut into pieces and weighed. 250g of the cut leaves were pulverized in an electric blender (Philip Electrical, UK), soaked in 2 litres of water for 3 hours and later filtered through a 1mm mesh sieve. The filtrate was made up to 10 litres with water, making a working dilution of 25,000ppm of the water extract of Aloe vera.

Study design

Albino Wistar rats of either sex (weighing 150-200g) were obtained from authorized animal house (Jamia Hamdard, Delhi, India). The animals were housed in cages under controlled conditions of temperature (25°C) and alternating 12 hour cycle of light and darkness. The animals had free access to standard rat pellet diet (Lipton India Ltd.) and tap water ad lib. After one week of acclimatization, the animals were considered suitable for study. These animals were divided into six groups of six animals each.

Group-I was given normal saline 2ml/100g per orally in addition to the standard rat pellet diet and tap water. Group-II was given 1 ml/kg of a 50% v/v solution of CCL₄ (Nice Chemicals Pvt. Ltd., Cochin) in olive oil intraperitoneally.

Group-III was given Liv.52 syrup (2 ml/kg) orally for 10 days followed by CCL₄ intraperitoneally as in Group-II. The Liv.52 was administered by gavage method with animals fasted 3-4 hours prior and 1 hour after administration to ensure proper absorption. CCL₄ dose was given concomitantly with the last (10th day) dose of Liv.52.

Group-IV received the test compound in the dose of 2ml/100g/day twice daily orally for the duration of 10 days followed by CCL₄ intraperitoneally as in Group-II.

Group-V was given the test compound as in group IV for the duration of 20 days followed by CCL₄ intraperitoneally on 20th day.

Groups-VI received the test compound as in group V for 30 days followed by CCL₄ intraperitoneally on 30th day. Every time the test compound was administered by gavage method with animals fasted 3-4 hours prior and 1 hour after administration to ensure proper absorption. Food was completely restricted after the administration of CCL₄ in
Groups II-VI. Animals of all the groups were fasted for 24 hours (during which duration water remained freely available) after which they were given anaesthesia, Ketamine (75 mg/kg) and Diazepam (10 mg/kg) intraperitoneally (Wixson et al., 1987). Blood was collected from the anaesthetized animals from retro-orbital plexus. After blood collection the animals were sacrificed to take out the liver for histopathological examination.

Biological study parameters

The collected blood, after a standing time of half an hour, was centrifuged in Remi R-8 centrifuge at 2500 rpm for 10 min. The serum so obtained was used to estimate the intended biochemical study parameters viz. Alanine transaminase (ALT) based on 2, 4-dinitrophenylhydrazine method (Reitman et al., 1957), Alkaline phosphatase (ALP) based on Kind and King’s method (Kind et al., 1954) and Total bilirubin based on modified Jendrassik and Grof’s method (Tietz et al., 1986).

Statistical significance was determined using Student- t Test. The difference in results between groups was considered as significant if P<0.05. The results are expressed as mean±SEM of six animals from each group (Ghosh 2008).

Histopathology

The liver was excised from the animals and washed with the normal saline. About one cm piece was cut and fixed in 10% neutral formalin for 12-24 hours. It was then dehydrated and cleared with ethanol and xylene respectively followed by embedding in paraffin wax from which blocks were prepared. Sections of 5µm thickness were prepared from the blocks using a microtome (Talib et al., 2007). These were processed in alcohol-xylene series and were stained with Harris haematoxylin and eosin stain (Clayden et al., 1971) and subjected to histopathological examination.

RESULTS

ALT level in normal saline treated group was 35.41±7.05 IU/l. It was found to be significantly increased (p<0.01) with administration of CCl₄ to 444.38±48.69 IU/l. Pretreatment with known hepatoprotective preparation Liv.52 significantly (p<0.01) limited the rise in ALT levels after CCl₄ administration to 114.43±21.99 IU/l. Administration of aqueous extract of Aloe vera exhibited dose dependant limitation of
ALT rise after CCl$_4$ administration. Although the doses of 2 ml/100g for 10 days and 2 ml/100g for 20 days showed a significant limitation (p<0.01) of ALT rise (298.48±27.56 IU/l and 194.92±13.38 IU/l respectively) when compared to CCl$_4$ treated group, it did not match the efficacy of Liv.52 treated group. However, in dose of 2 ml/100g for 30 days, the Aloe vera extract showed much more efficacy, in limiting the ALT rise, to 117.63±12.67 IU/l, which was highly significant (p<0.01) (Table.1, Fig.1)

A highly significant (p<0.01) rise in serum ALP levels was seen in CCl$_4$ treated group (554.22±64.92 IU/l) as compared to the normal saline treated group (76.74±10.76 IU/l). The rise in serum ALP was significantly low (p<0.01) in Liv.52 treated group (212.09±20.42 IU/l) as compared to only CCl$_4$ treated group.

The effect of Aloe vera treatment on serum ALP levels was dose related and followed similar trends to those of ALT. As with ALT, the doses of 2ml/100g for 10 days and for 20 days produced significantly less (p<0.01) increments in serum ALP (394.76±17.93 and 290.22±20.06 IU/l respectively) as compared to the CCl$_4$ treated group. However, in dose of 2ml/100g for 30 days, Aloe vera showed significantly better (p<0.01) prevention in rise of ALP (195.34±17.87) (Table.1, Fig.2)

CCl$_4$ significantly increased (p<0.01) the serum bilirubin (2.77±0.55 mg/dl) as compared to normal saline treated group (0.29±0.17 mg/dl). Although each of the treated groups with either Liv.52 or Aloe vera displayed significantly low values of total bilirubin (p<0.01 for all) as compared to CCl$_4$ treated group, they failed to show any significant difference among themselves. The total bilirubin levels for Liv.52, Aloe vera 2 ml/100g for 10, 20, and 30 days were 0.31±0.17, 0.63±0.16, 0.45±0.16, and 0.27±0.05 mg/dl respectively. It is worth noting that the total bilirubin values for the treatment received groups (Aloe vera and Liv.52) were in the normal range (Normal range for total bilirubin in albino rats: 0-0.55 mg/dl. (Table.1, Fig.3)

Effect on histology

Histology of liver of normal saline treated group showed normal architecture. The hepatic cords and the sinusoids were well visible (Fig.4). Classical centrizonal necrosis
was seen in the CCl₄ treated group. The hepatocytes around the central vein were necrosed with no distinguishable nuclei (Fig.5). Liv.52 treated group revealed very mild signs of liver injury. Only difference from the normal saline treated group was the presence of inflammatory cells and constricted sinusoids indicating apparent hepatocyte swelling (Fig.6). The group treated with Aloe vera (2 ml/100g for 10 days) showed feathery degeneration in the centrizonal area which was the predominant histological feature of this group. Necrosis was absent (Fig.7). The predominant feature of the group treated with Aloe vera for 20 days was the presence of fatty changes in the centrizonal area. Necrosis was not seen (Fig.8). Almost normal hepatic lobule architecture was seen in the group treated with Aloe vera for 30 days, the only difference being the presence of constricted sinusoids, pointing towards mild hepatocyte swelling. No necrosis or inflammation was seen. (Fig.9)

**DISCUSSION**

Liver damage induced by CCl₄ is a commonly used model for the screening of hepatoprotective drugs (Slater, 1965). It is metabolically activated by the cytochrome P-450 dependent mixed oxidease in the endoplasmic reticulum to form trichloromethyl free radical (CCl₃) which combined with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation (Recknagel et al., 1976; Recknagel et al., 1983; DeGroot et al., 1986). These result in changes in structures of the endoplasmic reticulum and membranes of other organelles, loss of metabolic enzyme activation, reduction of protein synthesis and loss of glucose 6 phosphatase activation, leading to liver injury and elevated levels of transaminases, alkaline phosphatase, bilirubin etc. (Recknagel et al., 1973; Gravel et al., 1979; Wolf et al., 1980; Azri et al., 1992).

Serum alanine transaminase, and alkaline phophatase were found to be significantly elevated after CCl₄ administration (Table 1, Fig. 1 & 2) though the rise in bilirubin level was not to the same extent like ALP and ALT. (Visweswaram et al., 1994; Jayasekhar et al., 1997) This could be explained by the fact that bilirubin reaches at peak serum level in the second hour after CCl₄ administration and probably declines afterwards. (Kulcsar et al., 1997) Blood
collection in the present study was 24 hours after CCl₄ administration and thus, the serum bilirubin levels would have been on the decline (Table 1, Fig. 3). Further, histopathology showed severe centrilobular necrosis, hepatocyte necrosis, portal inflammation, inflammatory cells infiltration, and macro and micro-vesicular steatosis (Fig. 5). The preferential affection of zone 3 is attributed to the enzyme specificity and metabolic heterogeneity of hepatocytes as suggested by Gumucio (Gumucio 1989).

It was observed, that Liv.52 significantly suppressed the rise of ALT and ALP after CCl₄ challenge. It also normalized the bilirubin levels (Table 1, Fig. 1, 2, & 3). This biochemical protection was also reflected in the histology which showed only mild hepatocellular swelling with the presence of some inflammatory cells (Fig. 6).

Aloe vera exhibited dose dependant hepatoprotection, both biochemically and histologically (Table 1, Fig. 1, 2, 3, 7, 8 & 9). It is interesting to note that the Aloe vera extract (2ml/100g) for 30 days provided better results with ALP as compared to Liv.52. Same dose for same duration gave good protection from increasing ALT though level of protection was slightly less as compared to Liv.52. In all Aloe vera treated groups and Liv.52 treated group, the bilirubin levels were in normal range (Kulscar et al., 1997)

Since formation of free radicals by cytochrome P450 after metabolism of CCl₄ (Weber et al., 2003) has been implicated for lipid peroxidation mediated hepatocyte injury, the hepatoprotective mechanisms of Liv.52 has been ascribed to inhibitory effect on the microsomal enzymes so that generation of free radicals is limited. Liv.52 is also claimed to act as free radical scavengers thereby preventing lipid peroxidation. Another suggested mechanism of Liv.52 is its anti-oxidant property, on account of which it may exert an inhibitory effect on lipid peroxidation and stimulatory effect on hepatic regeneration as well (Wroblewski et al., 1955; Molander et al., 1957; Zelman et al., 1959)

Aloe vera leaves contain a range of biologically active compounds, the best studied being acetylated mannans, polymannans, anthraquinone C-glycosides,
anthrones and anthraquinones and various lectins (Boudreau et al., 2006; King et al., 1995; Eshun et al., 2004). The possible hepatoprotective mechanism of aqueous extract of Aloe vera is not clearly known. It is assumed that the effect of Aloe vera extract on liver protection may be due to several reasons. It may be related to glutathione-mediated detoxification. Aloe vera could have enhanced GSH status in cells and thereby afforded protection to hepatic cells from toxic damages.

The effects of silymarin, a proven hepatoprotective agent, are mainly attributable to its antioxidant and free radical scavenging properties (Muriel et al., 1990). Like silymarin, Aloe vera might also act as antioxidant and free radical scavenger.

Some still unexplained mechanisms of Aloe vera may be assumed to be involved in protecting liver from carbon tetrachloride induced toxicity. The hepatoprotective activity of Aloe vera aqueous extract was comparable in the dose of 2 ml/100g/day for 30 days to that with Liv.52 syrup (2 ml/kg) for 10 days. This fact does not undermine the efficacy of the Aloe vera aqueous extract because; the Liv.52 is a hydroalcoholic extract. Thereby, a hydroalcoholic extract of Aloe vera can be expected to deliver these results at much smaller doses and for less duration. Further the pharmacokinetic studies of Aloe vera are largely unknown. An elaborate investigation to explore the pharmacokinetic profile may lead to better efficacy and potency of Aloe vera at smaller doses.

CONCLUSION

It can be concluded from the study, that Aloe vera aqueous extract is a potent hepatoprotective agent. Also, since the safety profile of Aloe vera aqueous extract has been very encouraging in this study, direct human studies can be undertaken without any risk. Further studies of extract of Aloe vera could be extended for the isolation and structure determination of the hepatoprotective principle(s).
Effect of Liv.52 (2 ml/kg, po) and Aloe Vera (AV) in dose of 2 ml/100g/day (po) for different durations, on carbon tetrachloride (CCl4) induced changes in Serum Alanine Transaminase (ALT) (Mean ± SD) (n=6).

▲ p<0.001 as compared to NS treated group.
★ p<0.01 as compared to CCl4 treated group

NS: Normal Saline
Figure 2

Effect of Liv.52 (2 ml/kg, po) and Aloe Vera in dose of 2 ml/100g/day (po) for different durations, on carbon tetrachloride (CCl4) induced changes in Serum Alkaline Phosphatase (Mean ± SD) (n=6).

▲ p<0.001 as compared to NS treated group

★ p<0.001 as compared to CCl4 treated group.

NS: Normal Saline
Effect of Liv.52 (2 ml/kg, po) and Aloe Vera (AV) in dose of 2 ml/100g/day (po) for different durations, on carbon tetrachloride (CCl4) induced changes in Serum Total Bilirubin (Mean ± SD) (n=6).

▲ p<0.001 as compared to NS treated group.
★ p<0.01 as compared to CCl4 treated group

NS: Normal Saline
Figure 4 Microscopic features of the liver of Normal Saline treated group (Gr I). Normal hepatic lobule architecture is seen. Hepatocytes and their nuclei are well visible. (H & E stain x400)

CV: Central Vein. Arrow: Sinusoid.

Figure 5 Microscopic features of the liver of CCl4 treated group (Gr II). Extensive centronzonal necrosis is seen. Only cellular debris is seen and no hepatocytes with nuclei are discernible. (H & E stain x400) CV: Central Vein.
Figure 6 Microscopic features of the liver of Liv.52 treated group (Gr III). Mild hepatocyte swelling is present as indicated by constricted sinusoids. Inflammatory cells are seen mostly around central veins. Necrosis is absent (H & E stain x400). Solid arrows: Sinusoids, Outline arrows: Inflammatory cells

Figure 7 Microscopic features of the liver of group treated with Aloe vera aqueous extract 2ml/100g/d for 10 days (Gr IV). Remarkable feathery degeneration of hepatocytes is seen around the central vein. Further away from the central vein, cells with fatty changes are seen. Necrosis is not seen. (H & E stain x400) CV: Central Vein.
Figure 8 Microscopic features of the liver of group treated with Aloe vera aqueous extract 2ml/100g/d for 20 days (Gr V). Centrizonal area is conspicuous by the presence of hepatocytes showing fatty changes. Necrosis is not seen (H & E stain x400). CV: Central Vein. Arrows: showing feathery degeneration.

Figure 9 Microscopic features of the liver of group treated with Aloe vera aqueous extract 2ml/100g/d for 30 days (Gr VI). Constricted sinusoids (solid arrows) are seen pointing to mild hepatocyte swelling. Necrosis is absent. (H & E stain x400) CV: Central vein
Table-1

Effect of Liv.52 (2 ml/kg) and Aloe Vera (AV) in dose of 2 ml/100g/day for different durations, on carbon tetrachloride (CCl4) induced changes in various biochemical parameters (Mean ± SD) (n=6).

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>ALANINE TRANSAMINASE (IU/l)</th>
<th>ALKALINE PHOSPHATASE (IU/l)</th>
<th>TOTAL BILIRUBIN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Saline</td>
<td>35.41±7.05</td>
<td>76.74±10.76</td>
<td>0.29±0.17</td>
</tr>
<tr>
<td>Carbon Tetrachloride (1ml/kg, ip)</td>
<td>444.38±48.69**</td>
<td>554.22±64.92**</td>
<td>2.77±0.55**</td>
</tr>
<tr>
<td>Liv.52 (2ml/kg), for 10 days + CCl4 on 10th day</td>
<td>114.43±21.99*</td>
<td>212.09±20.42*</td>
<td>0.31±0.17*</td>
</tr>
<tr>
<td>AV 2 ml/100g/d, for 10 days + CCl4 on 10th day</td>
<td>298.48±27.56*</td>
<td>394.76±17.93*</td>
<td>0.63±0.16*</td>
</tr>
<tr>
<td>AV 2 ml/100g/d, for 20 days + CCl4 on 20th day</td>
<td>194.92±13.38*</td>
<td>290.22±20.06*</td>
<td>0.45±0.16*</td>
</tr>
<tr>
<td>AV 2 ml/100g/d, for 30 days + CCl4 on 30th day</td>
<td>117.63±12.67*</td>
<td>195.34±17.87*</td>
<td>0.27±0.05*</td>
</tr>
</tbody>
</table>

**p<0.001 as compared to normal saline treated group.
*p<0.001 as compared to CCl4 treated group.

REFRENCES

4. [http://www.karinya.com/liver.htm](http://www.karinya.com/liver.htm)


