



Research Article

HEPATOPROTECTIVE AND HISTOPATHOLOGICAL ACTIVITY OF ETHANOL AND AQUEOUS EXTRACTS OF STEM OF ALOE VERA LINN (GHEE GANGWAR) AGAINST PARACETAMOL-INDUCED LIVER DAMAGE IN RATS

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Received October 27, 2015; Accepted December 20, 2015; Published January 08, 2016;

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Cite This Article:Hena, Tiwari, P., Srivastava, M., Ghoshal, S.(2016). Hepatoprotective and histopathological Activity of Ethanol and Aqueous extracts of stem of Aloe vera Linn (Ghee gangwar) against paracetamol-induced liver damage in rats. International Journal of Pharmacy & Bio-Sciences, 1(1).1-7

ABSTRACT

Aim of the study: Aloe vera is a medicinal plant widely distributed in the various parts of India. Aloe Vera is a major medicinal plant when it comes to treating and protecting the skin. Used externally, it is very effective on burns and sunburn, as well as a variety of skin diseases (eczema, pruritus, psoriasis, acne) – it is extremely constructive and protective. Root contains resinous matter and fat. It is used as Stomachic hepato-biliary affections etc. The present research was aimed to evaluate the potential hepatoprotective activity of ethanol and aqueous extracts of stem using *in vivo* models to validate the folkloric use of the plant.

Materials and methods:The hepatoprotective activity of the ethanol and aqueous extracts of stem were studied on male albino wistar rats, liver damage induced by paracetamol (2.5gm/kg, p.o.) by monitoring biochemical parameters. Various biochemical parameters were studied to evaluate the hepatoprotective activity of ethanol and aqueous extracts in serum like glutamic pyruvic transaminase (sGPT), serum glutamic oxaloacetic transaminase (sGOT) and serum alkaline phosphatase (sALP), total bilirubin (sB), total protein, total cholesterol and histopathological changes in liver were also studied along with silymarin (100mg/kg, p.o.) as standard hepatoprotective agents were determined to assess the effect of the ethanol and aqueous extracts of stem of *Aloe vera* (100 and 200 mg/kg) on the paracetamol induced hepatic damage.

Results:The phytochemical investigation of the extracts showed presence of carbohydrates, proteins, steroids and flavonoids. Pre-treatment of the rats with ethanol and aqueous extracts prior to paracetamol administration caused a significant reduction in the values of sGOT, sGPT, sALP and sB (P<0.01) almost comparable to the silymarin. The hepatoprotective was confirmed by histopathological examination of the liver tissue of control and treated animal.



Conclusions:The results indicate that this plant possesses potential hepatoprotective properties and has therapeutic potential for the treatment of liver diseases.

KEYWORDS:Aloe vera Linn. Paracetamol; hepatoprotective activity; silymarin; histopathology

INTRODUCTION

The history of herbal medicines is as old as human civilization. Medicinal plants have main and cheap source of unique phytoconstituents, they are used extensively for the development of new drugs against various diseases and disorders.[1, 2] Herbal drugs play an important role in treatment of various ailments including hepatopathy (liver ailments). Medicinal herbs are widely used in the treatment of liver diseases like hepatitis, cirrhosis, and loss of appetite, some medicinal herbs have proven hepatoprotective potential. Silymarin, a flavonolignan mixture extracted from the milk thistle (*Silybummarianum*) is a popular remedy for hepatic diseases. [3]

Among the medicinal plants, *Aloe vera* (Family – Xanthorrhoeaceae) (known as Ghee gangwar) is a useful Indian medicinal plant which has been credited with therapeutic properties to treat several diseases. *Aloe vera* is found in various parts of India. *Aloe vera* is having very short-stemmed succulent plant growing to 60–100 cm (24–39 in) tall, spreading by offsets. The plant has triangular, fleshy leaves with serrated edges, yellow tubular flower and fruits that contain numerous seeds. [4] However, scientific literature data supporting the folkloric use of the *Aloe vera* in liver diseases are not available and its tentative mechanism(s) are still unknown.

On the basis of literature review and tribal information gathered from Allahabad interior area, Uttar Pradesh that the plant *Aloe vera* (Ghee gangwar) has reported the use of the stem for the management of hepatotoxicity. Hence, the objective of this study was to ascertain the scientific basis for the use of *Aloe vera* Linn. (Xanthorrhoeaceae) in the management of hepatotoxicity using paracetamol induced hepatotoxicity rats.

MATERIALS AND METHODS

Collection Of Plant Material And Extraction

The stem of *Aloe vera* was collected from the local area of Allahabad interior area, Uttar Pradesh, India in the month of March 2015.

The stem was dried under shade, powdered with a mechanical grinder and passed through a 40-mesh sieve. The successive solvent cold extraction

method used to obtain various extracts including petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts. The solvents were removed from the extracts under reduced pressure by using a rotary vacuum evaporator (Buchi model, Jyoti Lab, Gwalior, India). The percentage of yield of extracts was noted. The brownish extract was obtained and is dissolved in their respective solvents for pharmacological studies.

Preliminary phytochemical screening

The ethanol and aqueous extracts of stem of *Aloe vera* Linn was screened for the presence of various phytoconstituents like steroids, alkaloids, glycosides, flavonoids, carbohydrates, amino acids, proteins and phenol compounds.[5]

Animals

Healthy, adult Albino Wistar rats (180-200gm) of either sex were purchased from the National Center for Laboratory Animal sciences, Hyderabad used for study. Housed individually in polypropylene cages, maintained under standard conditions (12 h light; and 12 h dark cycle; 23±2°C, 50± 5%, relative humidity), they were fed with standard rat pellet diet (Hindustan Lever Ltd; Mumbai, India) and were ad libitum. The Institutional Animal Ethics Committee approved the study.

Acute toxicity study

The acute oral toxicity study has to be carried out as per the guidelines set by OECD, revised draft guidelines 423, received from CPCSEA, ministry of social justice and empowerment, Govt. of India. The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation canula. In the unusual circumstance that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. [6]

In vivo Hepatoprotective activity

The rats have to be randomized into seven groups comprising of six animals in each groups as given below.

Group I: Normal control rats were given Tween 80 for 4 days.

Group II: Hepatotoxicity control rats have been given Tween 80 for 4 days followed by paracetamol (2.5gm/kg, p.o.) on 3rd day.

Group III: Rats have been given Silymarin (100mg/kg, p.o.) for 4 days followed by paracetamol (2.5gm/kg, p.o.) on 3rd day.

Group IV: Test rats have been given ethanol extract of stem of *Aloe vera* (100mg/kg, p.o.) followed by paracetamol (2.5gm/kg, p.o.) on 3rd day.

Group V: Test rats have been given ethanol extract of stem of *Aloe vera* (200mg/kg, p.o.) followed by paracetamol (2.5gm/kg, p.o.) on 3rd day.

Group VI: Test rats have been given aqueous extract of stem of *Aloe vera* (100mg/kg, p.o.) followed by paracetamol (2.5gm/kg, p.o.) on 3rd day

Group VII: Test rats have been given aqueous extract of stem of *Aloe vera* (200mg/kg, p.o.) followed by paracetamol (2.5gm/kg, p.o.) on 3rd day. [7]

Assessment Of Hepatoprotective Activity

At the end of 5th day, blood was collected by heart puncture and serum was separated for the estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, total cholesterol, etc. and the liver was isolated from the rats of all the groups and kept in 10% formalin solution and hence send for histopathological investigation. [7]

Statistical Analysis

Hepatoprotective activity:The data was represented as mean ± SEM. Results was analyzed by one way ANOVA followed by Dunnett’s multiple comparison tests using Graph pad instat 3.0 software. Results were expressed as the mean ± S.E.M. for statistical analysis of the data group means, were compared by one-way analysis of variance (ANOVA) followed by Tukey’s post-test for multiple comparisons. p < 0.001 was considered to be statistically significant.

Table 1: Preliminary phytochemical study of the extracts of stems of Aloe vera

Phytoconstituents/ Extracts	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Aqueous
Alkaloids	-	+	-	+	-
Glycosides	-	+	+	+	+
Flavonoids	-	+	+	+	+
Steroids	-	-	-	+	-
Phenolics and Tannins	-	-	-	+	+
Fixed oils	-	-	-	-	-

+ =Presence; -=Absence



Table 2: Effect of *Aloe vera* on serum marker enzymes (SGOT, SGPT, and ALP), Total bilirubin, total protein and total cholesterol on paracetamol induced hepatotoxicity in rats

Groups	Treatment	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total Bilirubin (mg/dl)	Total Protein (gm/dl)	Total cholesterol (Mg %)
I	Normal control	158.28 ± 0.0601**	88.28 ± 0.0601**	166.26 ± 0.0667**	0.655 ±0.0076**	8.85 ± 0.0076**	10.66 ± 0.0058**
II	Paracetamol 2.5 mg/kg	246.26 ±0.0667	128.26 ± 0.0667	242.21 ±0.0477	6.23 ± 0.0097	4.62 ±0.0058	30.22 ±0.0058
III	Silymarin 100 mg/kg.	162.22 ± 0.0477**	91.21 ± 0.0477**	178.26 ± 0.0667**	0.735 ± 0.0076**	7.848 ± 0.0048**	15.158 ± 0.0065**
IV	Ethanol 100 mg/kg + Paracetamol	171.28 ± 0.0477**	93.28 ± 0.0477**	179.3 ± 0.0577**	0.7433 ± 0.0088**	7.213 ± 0.0049**	17.53 ± 0.0058**
V	Ethanol 200 mg/kg + Paracetamol	174.26 ±0.0667**	98.26 ± 0.0667**	182.28 ± 0.0477**	0.845 ± 0.0076**	6.9566 ± 0.0071**	18.213 ± 0.0061**
VI	Aqueous 100 mg/kg + Paracetamol	164.28 ± 0.0601**	92.28 ± 0.0601**	172.28 ± 0.0601**	0.725 ± 0.0076**	7.82 ± 0.0058**	16.513 ± 0.0049**
VII	Aqueous 200 mg/kg + Paracetamol	168.3 ± 0.0577**	94.3 ± 0.0577**	178.28 ± 0.0601**	0.7516 ± 0.0060**	7.66 ± 0.0058**	17.615 ± 0.0056**

Values are expressed as mean ± SEM for (n=6) rats in each group, when compared to control **p<0.01

Histopathological studies

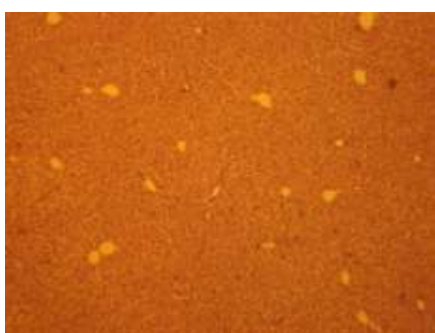


Fig 1:Normal group showing normal cellular architecture with distinct sinusoidal space and central vein

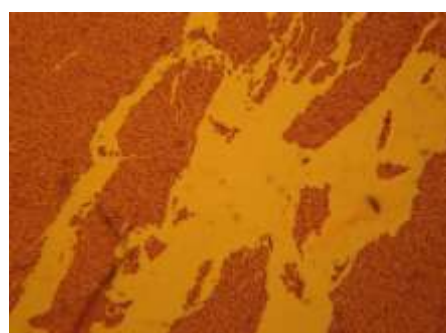


Fig 2:Toxic control group showing severe necrosis of hepatocytes





Fig 3: Standard Silymarin (100mg/kg, p.o.) treated group showing less disarrangement of hepatocytes as well as marked regeneration activity

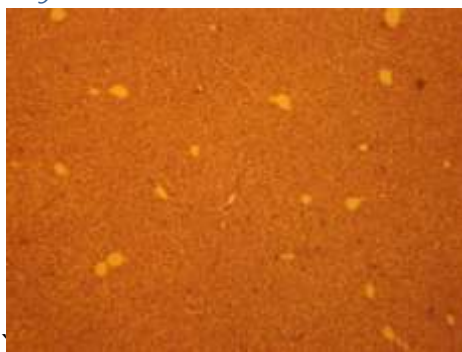


Fig 4: Ethanol highdose (200 mg/kg) showing normal architecture with moderate hepatocyte generation

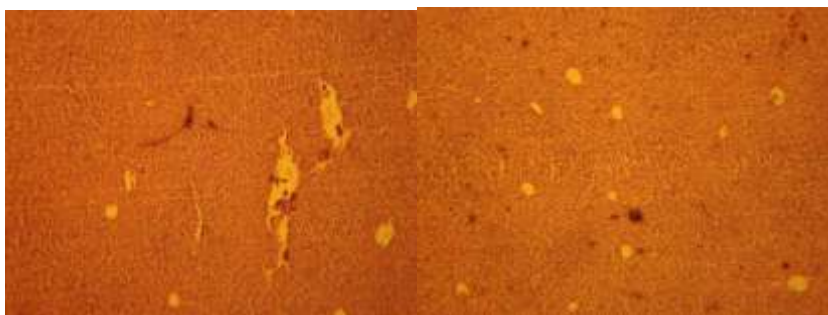


Fig 5: Ethanol low dose (100mg/kg) showing normal architecture with moderate degeneration.

Fig 6: Aqueous high dose (200mg/kg) showing normal architecture with hepatocyte moderate hepatocyte degeneration.



Fig 7: Aqueous low dose (100mg/kg) showing normal architecture with moderate hepatocyte degeneration



RESULTS

Table 1 shows the results of Preliminary study was performed on the aqueous and ethanol extracts of the stem of *Aloe vera* and the presence of various phytoconstituents such as alkaloids, glycosides, flavonoids, steroids, fixed oils, Phenolics and tannins were determined.

Hepatoprotective activity

Table 2 shows the results of hepatoprotective activity of ethanol and aqueous extracts of stem of *Aloe vera* on paracetamol induced hepatotoxicity in rats. The hepatic enzymes SGOT, SGPT, ALP, bilirubin, total protein and total cholesterol in serum was significantly increased in paracetamol treated animals when compared to normal. The ethanol and aqueous extracts of stem of *Aloe vera* low and high dose significantly reversed the levels of SGOT, SGPT, ALP, bilirubin, total protein and total cholesterol respectively when compared to paracetamol alone treated rats. So the animals treated with ethanol and aqueous extracts of stem of *Aloe vera* showed statistically significant ($p < 0.01$) protection against paracetamol induced hepatotoxicity in rats, which is comparable to the reference compound Silymarin. Thus Silymarin (100mg/kg) treated animals showed significant decrease in SGOT, SGPT, ALP, bilirubin, total protein and total cholesterol levels.

Histopathological Study

The histopathology study of liver was also performed which showed hepatoprotective effect. The hepatoprotective effect of *Aloe vera* stem was confirmed by histopathological examination of the liver tissue of control and treated animals. The histological architecture of liver sections of healthy rats showed normal cellular architecture with distinct hepatic cells and sinusoidal space (Fig^[1]). In the liver section of the rats intoxicated with paracetamol (Fig^[2]), there was disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis, while rats treated with silymarin and intoxicated with paracetamol showed less disarrangement and degeneration of hepatocyte (Fig^[3]). The histopathological profile of the rat treated with ethanol extract showed no visible changes confirming the safety of the extract at selected higher and lower doses (Fig^[4, 5]) and the liver section of the rats treated with higher and lower doses of aqueous extracts and intoxicated with paracetamol showed moderate hepatoprotective activity (Fig^[6, 7]).

DISCUSSION

Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthenes.

Here we have demonstrated for the first time that *Aloe vera* ethanol and aqueous extract has an important hepatoprotective effect against paracetamol. This is particularly important in view of the fact that the treatments of acute human intoxications with paracetamol are limited and frequently not effective and relies basically on the use of *N*-acetylcysteine. [8, 9] Paracetamol is a well-

known antipyretic and analgesic agent, which is safe in therapeutic doses, but can produce fatal hepatic necrosis in experimental animals and humans [10, 11] and is employed as an experimental hepatotoxic agent. An obvious sign of hepatic injury is the leaking of cellular enzymes into the plasma due to the disturbance caused in the transport functions of hepatocyte. [12] The estimation of enzymes in the serum is a useful quantitative biochemical marker of the extent and type of hepatocellular damage. Assessment of liver function can be made by estimating the activities of serum ALT, AST, ALP and Bilirubin which are enzymes originally present higher concentration in cytoplasm.

The rat treated with an overdose of paracetamol developed significant hepatic damage, which was observed by a substantial increase in the concentration of serum hepatic enzymes (sGPT and sGOT). Biochemical parameters demonstrate significant increase of serum enzymes in the toxic control groups in the present study. Histopathological profile also reveals a major damage in the same groups. Thus, it clearly states that, toxicity is due to either of the above mechanisms such as depletion of glutathione store or free radical generation or lipid peroxidation.

Administration of *Aloe vera* stem ethanol and aqueous extracts (100 mg/kg and 200mg/kg p.o.) after paracetamol treatment resulted in a significant reduction ($p < 0.05$) of paracetamol-induced elevation of sGPT and sGOT and appears to be protective in reducing the injurious effect of paracetamol.

Bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte. Decrease in serum bilirubin after treatment with extract in liver damage induced by paracetamol, indicated the effectiveness of the extract in normal functional status of the liver.

In present study the histopathological studies also shows normal, toxic, toxic control (silymarin treated), ethanol higher and lower dose treated, and aqueous higher and lower dose treated hepatocytes of liver.

In conclusions, the ethanol and aqueous extracts of stem of *Aloe vera* exhibited protective effect against paracetamol-induced hepatotoxicity and possess anti-lipid peroxidative and free radical scavenging activities. The result supports the use of the plant as

described in folkmedicine, that the plant root can be used to treat liver and gastric disorders.

Further studies are required to isolate the active constituents involved in the hepatoprotective activity of the plant.

ACKNOWLEDGEMENT

I am very much thankful to my guide Ms. Pallavi Tiwari and Shambhunath Institute of Pharmacy, Allahabad (U.P.) for providing best lab facilities necessary for completion of research project.

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