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Research Article

STUDY OF INNER LEAF JUICE OF *ALOE BARBADENSIS* IN RESTORING THE INTEGRITY OF HEPATOCYTES AGAINST EXPERIMENTALLY INDUCED LIVER INSULT AND LIVER FIBROSIS

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ABSTRACT:

Hepatocytes are the predominant liver cells which are responsible for the multifold functions of the liver and are susceptible to injury by the drugs like paracetamol. Herbal drugs play an important role in the cure of liver diseases. Aloe vera (Aloe barbadensis) has been used for many centuries for it's curative and therapeutic properties, and health benefits associated have been attributed to the polysaccharides contained in the gel of the leaves. The purpose of present study was to evaluate the hepatocurative effect of aqueous extracts of polysaccharide fractions of Aloe barbadensis against paracetamol induced liver injury and to find out the dose of the fraction at which maximum restoration of integrity of hepatocytes occur. Polysaccharide fractions were identified and isolated by mass spectrometry and column chromatography, respectively. Hepatotoxicity was induced with a single dose of paracetamol 3 g/kg, orally. N-acetylcysteine was used as a standard drug. The hepatocurative effect of the fractions was studied by biochemical parameters like SGPT, MDA, GSH, bilirubin and albumin. Animals were divided into seven groups. The first three groups received normal, disease control and standard respectively. Fraction I was given at doses of 250 mg/kg and 500 mg/kg, orally to group four and five respectively. They did not show any significant effect in restoring the liver enzymes. Fraction II was given at doses of 250 mg/kg and 500 mg/kg, orally to last two groups respectively. All the animals were treated for seven days. Treatment with fraction II at a dose of 500mg/kg significantly (P<0.001) restored the levels of SGPT, MDA, GSH, bilirubin and albumin and showed that the fraction had maximum hepatocurative activity.

Key words: *Aloe barbadensis, hepatocurative activity, polysaccharide fractions*

INTRODUCTION

The liver is a vital and largest organ having wide range of functions. The multifold functions of liver are due to the presence of predominant liver cells called hepatocyte.

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They play a vital role in clearing and transforming the chemicals but some medicinal agents like paracetamol (acetaminophen) can damage the liver by directly damaging the hepatocyte [1]. The normal function of hepatocytes or liver damage is indicated by some biochemical markers such as bilirubin, alanine transferase and alkaline phosphatase (ALP).

Paracetamol is used as analgesic and antipyretic. It is well tolerated and has a low

incidence of gastrointestinal side effects. At therapeutic doses, it is mostly conjugated in the liver with glucuronide or sulphate and is metabolized to a highly reactive intermediate NAPQI (N-acetyl-p-benzoquinone imine) through the Cyt P-450 system. However, when taken in large doses, the amount of NAPQI formed is sufficient to deplete hepatic GSH, leading to hepatic necrosis localized in the centrilobular areas of the hepatic lobules [2]. The depletion of GSH makes the hepatocytes more susceptible to cell death caused by reactive oxygen species and leads to apoptosis [3].

Herbal drugs play an important role in the cure of liver diseases. There is a lack of hepatoprotective drugs in allopathic medical practice, which can effectively cure liver diseases, but clinical trials are required to assess the role of medicinal herbs as therapeutic agents [4]. *Aloe vera* (*Aloe barbadensis*) is a member of *Liliaceae* family and has been used for many centuries for it's curative and therapeutic properties [5]. Many of the health benefits associated with *Aloe vera* have been attributed to the Polysachharides contained in the gel of the leaves. *Aloe barbadensis* gel has been demonstrated to have liver protective i.e., hepatoprotective effect in rats [1]. The phytochemical constituents of *Aloe vera* were studied for the hepatoprotective activity and the results of the study justified the use of *Aloe vera* polysaccharides as a hepatoprotective agent [6].

The molecular weight of *Aloe vera* polysaccharides ranges from 1,00,000 to 5,00,000 daltons and many research works suggested the existence of polysaccharide fractions. Thus, we planned this study to validate the hepatocurative activity of these variegated polysaccharide fractions of *Aloe vera* against paracetamol (PCT) induced liver damage, by performing an assay of serum bilirubin, serum albumin, SGPT, GSH and MDA, and liver histopathology.

MATERIALS AND METHODS

Collection of plant material, Extraction

Freshly cut leaves of *Aloe barbadensis* were collected from Arogya Rama Herbals, Shameerpet village, Hyderabad of Andhra Pradesh, India and botanically identified and authenticated by B. Rameshwar Reddy. A voucher specimen has been deposited at the herbarium of the Institute. The authenticated leaves were washed to remove earthy material and allowed to drain the juice overnight. After draining of the juice, epidermis of the leaves was removed, the gel was separated and subjected to homogenization. The supernatant was collected and subjected to phytochemical screening. It was allowed to dehydrate at 50^{o}c until the moisture was completely removed from the gel and the dried extract was collected. It was then used for analytical studies to separate individual polysaccharide fractions by TLC, HPLC and Column chromatography. The dried purified fractions obtained after column chromatography were used for hepatocurative studies.

Experimental animals

Healthy male wistar albino rats weighing 150-200 gm were used in the present study. They were housed in individual polypropylene cages under standard laboratory conditions of light, temperature (22±2^{o}c) and relative humidity (55%). They were fed with standard rat pellet diet and free access to water *ad libitum* [7]. The experimental protocol was approved by the Institutional Animal Ethics Committee of CMR College of Pharmacy, Medchal, Hyderabad-501401.

Hepatocurative study

Animals were divided into seven groups containing six animals each. Group I was kept as a control and was given 0.1 ml of 2% gum acacia for seven days. Group II was kept as a disease control in which hepatotoxicity was induced by single dose of paracetamol (3 g/kg, *p.o*). Group III served as a standard and received N-acetyl cysteine (450 mg/kg, *p.o*) for seven days, once daily. Group IV and V received low dose (250 mg/kg, *p.o*) and high dose (500 mg/kg, *p.o*) of polysaccharide fraction I respectively, once daily for seven days. Group VI and VII received low dose

(250 mg/kg, *p.o*) and high dose (500 mg/kg, *p.o*) of polysaccharide fraction II respectively for seven days, once daily. All the animals of Group III-VII received single dose of paracetamol (3 g/kg, *p.o*) one hour before the test dose on the first day. Dosing was given to all the animals at the same time for seven days.

Blood chemistry

Blood samples were collected by retro orbital puncture, 24 h after the last dose i.e., on the 8th day. The blood samples were allowed to clot for 60 min at room temperature. Serum was separated by centrifugation at 4000 rpm for 15 min and used for the estimation of serum parameters like SGPT, serum bilirubin and serum albumin.

Preparation of liver homogenate

All the animals were sacrificed by spinal dislocation and livers were quickly excised, freed from any adhering tissues, washed and perfused with chilled normal saline, minced and homogenized in ice bath [2] using homogenizer in chilled 50 mM phosphate buffer (pH 7.4) to obtain 10% liver homogenate for the estimation of (GSH) glutathione [8] and lipid peroxidation [9] by using standard methods. A part of the homogenate was taken and mixed with equal volume of 10% trichloroacetic acid (TCA) and centrifuged at 4000 rpm for 15 min at 4^{o}c and the supernatant i.e., clear solution was collected into another tube and was further used for the determination of parameters like MDA and GSH.

Histopathology

From all the animals used for the study, a small portion of liver tissue was fixed in 10% formalin for histopathological assessment. The tissue was processed and embedded in paraffin wax to obtain thin sections of 3 µm and eosin stained sections [10].

Statistical analysis

Statistical analysis was done by one way ANOVA followed by Dunnett's post analysis using Graph pad prism version 5.0, USA. All the values were presented as the mean±SEM. The data obtained was analysed for P-value [11]. P-value < 0.05 was taken as the criterion of significance and P-value < 0.001 was considered highly significant.

RESULTS

Biochemical analysis

Effect of *Aloe vera* fractions Ia, Ib, IIa and IIb on various enzyme levels in individual groups were expressed in terms of their mean±sem values.

- Effect of *Aloe vera* fractions on the levels of SGPT in different groups was given in the Table 1 and represented in Fig.1.
- Effect of *Aloe vera* fractions on the levels of Total Bilirubin in different groups was given in Table 2 and represented in Fig.2.
- Effect of *Aloe vera* fractions on the levels of Direct Bilirubin in different groups was given in Table 3 and represented in Fig.3.
- Effect of *Aloe vera* fractions on the levels of Albumin in different groups was given in Table 4 and represented in Fig.4.
- Effect of *Aloe vera* fractions on the levels of GSH in different groups was given in Table 5 and represented in Fig.5.
- Effect of *Aloe vera* fractions on the levels of MDA in different groups was given in Table 6 and represented in Fig.6.

Table 1: Effect of Aloe vera fractions on levels of SGPT

S. No	Group	Mean ± SEM (IU/L)
01.	Control	25.05 ± 1.048***
02.	Disease Control	74.85 ± 2.542
03.	Standard	30.8 ± 0.818***
04.	Fraction Ia	69.31 ± 2.474
05.	Fraction Ib	67.25 ± 2.246*
06.	Fraction IIa	62.20 ± 1.980***
07.	Fraction IIb	41.35 ± 1.923***

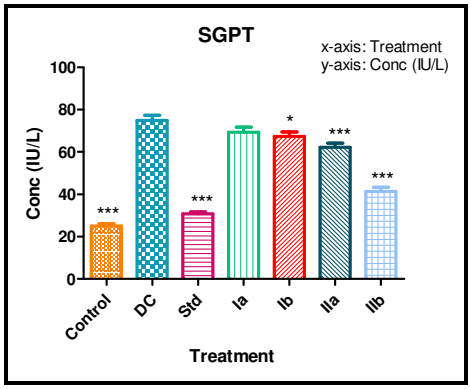


Fig.1:Effect of *Aloe vera* fractions on levels of SGPT

Table 2: Effect of Aloe vera fractions on levels of Total Bilirubin

S. No	Group	Mean ± SEM
01.	Control	1.11± 0.048***
02.	Disease Control	1.428 ± 0.015
03.	Standard	1.14 ± 0.046***
04.	Fraction Ia	1.353 ± 0.013
05.	Fraction Ib	1.326 ± 0.007
06.	Fraction IIa	1.301 ± 0.008*
07.	Fraction IIb	1.185 ± 0.037***

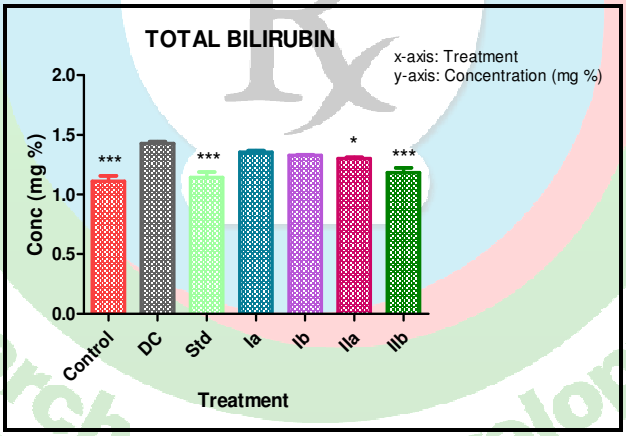


Fig.2: Effect of *Aloe vera* fractions on levels of Total bilirubin

Table 3: Effect of Aloe vera fractions on levels of Direct Bilirubin

S. No	Group	Mean ± SEM
01.	Control	0.221 ± 0.019***
02.	Disease Control	0.488 ± 0.021
03.	Standard	0.241 ± 0.020***
04.	Fraction Ia	0.445 ± 0.014
05.	Fraction Ib	0.411 ± 0.017*
06.	Fraction IIa	0.360 ± 0.017**
07.	Fraction IIb	0.271 ± 0.016***

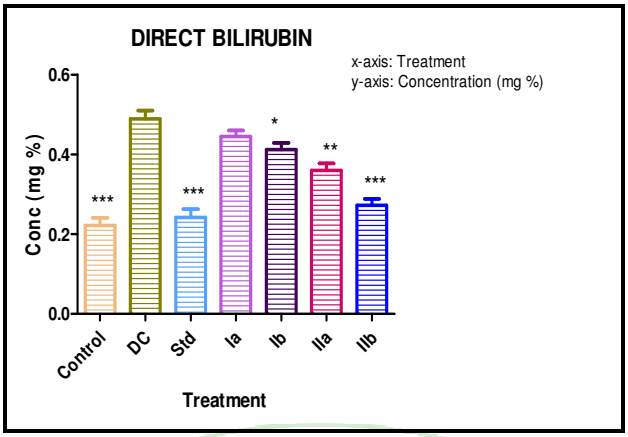


Fig.3: Effect of *Aloe vera* fractions on levels of Direct Bilirubin

Table 4: Effect of Aloe vera fractions on levels of Albumin

S. No	*Group*	*Mean ± SEM*
01.	Control	3.613 ± 0.215***
02.	Disease Control	1.748 ± 0.007
03.	Standard	3.496 ± 0.216***
04.	Fraction Ia	1.926 ± 0.005
05.	Fraction Ib	2.231 ± 0.014
06.	Fraction IIa	2.480 ± 0.024**
07.	Fraction IIb	3.275 ± 0.194***

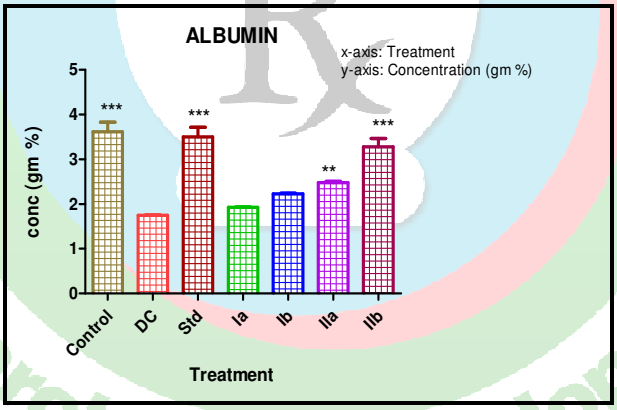


Fig.4: Effect of *Aloe vera* fractions on levels of Albumin

Table 5: Effect of Aloe vera fractions on levels of GSH

S. No	*Group*	*Mean ± SEM*
01.	Control	1.319 ± 0.045***
02.	Disease Control	0.375 ± 0.012
03.	Standard	1.330 ± 0.048***
04.	Fraction Ia	0.418 ± 0.006
05.	Fraction Ib	0.481 ± 0.010
06.	Fraction Iia	0.662 ± 0.027**
07.	Fraction Iib	1.045 ± 0.026***

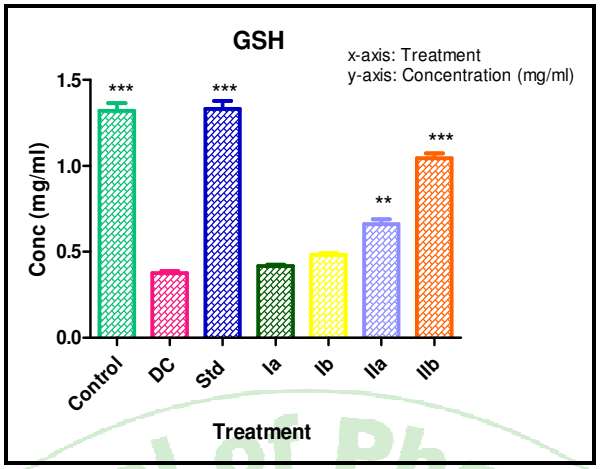


Fig.5:Effect of *Aloe vera* fractions on levels of GSH

Table 6: Effcet of Aloe vera fractions on levels of MDA

S. No	*Group*	*Mean ± SEM*
01.	Control	0.180 ± 0.021***
02.	Disease Control	0.495 ± 0.019
03.	Standard	0.011 ± 0.019***
04.	Fraction Ia	0.418 ± 0.008
05.	Fraction Ib	0.377 ± 0.009
06.	Fraction Iia	0.280 ± 0.006***
07.	Fraction Iib	0.196 ± 0.014***

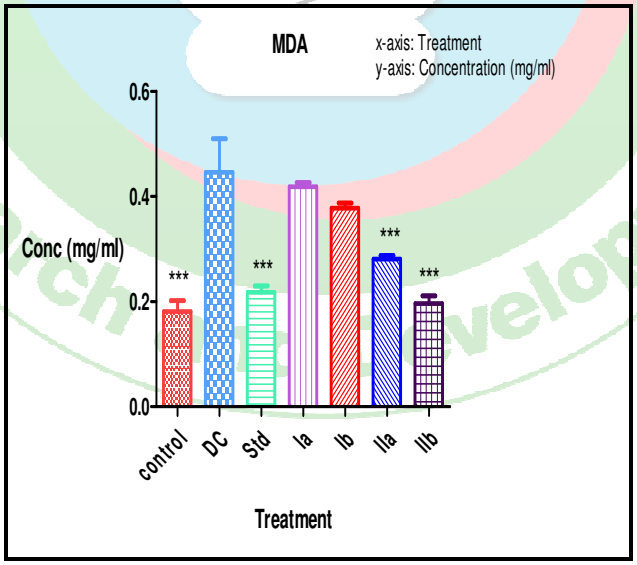
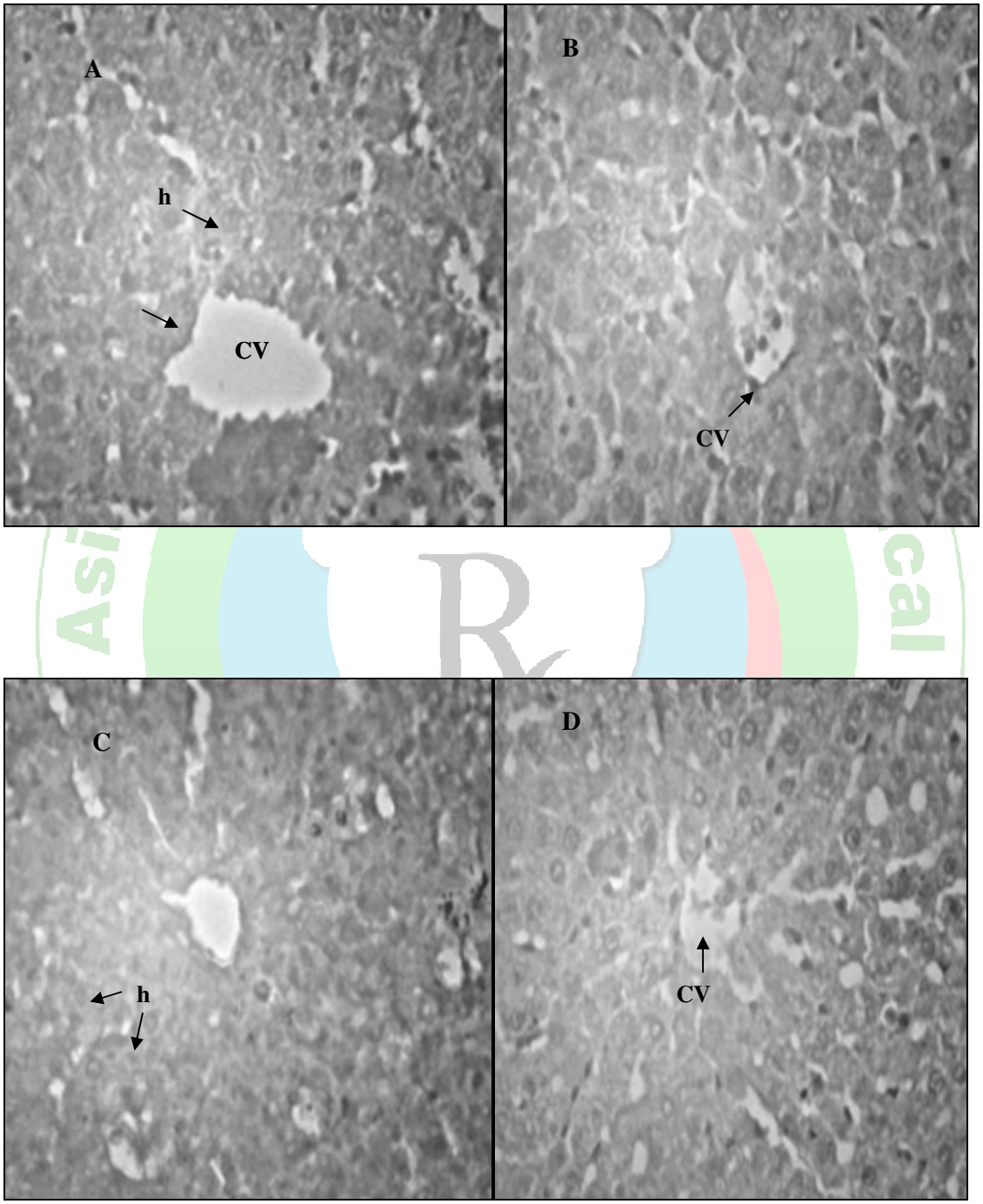


Fig.6: Effect of *Aloe vera* fractions on levels of MDA

Histopathological findings

Photomicrographs of liver sections of rats of various groups with their histological structure were shown in Fig.7.



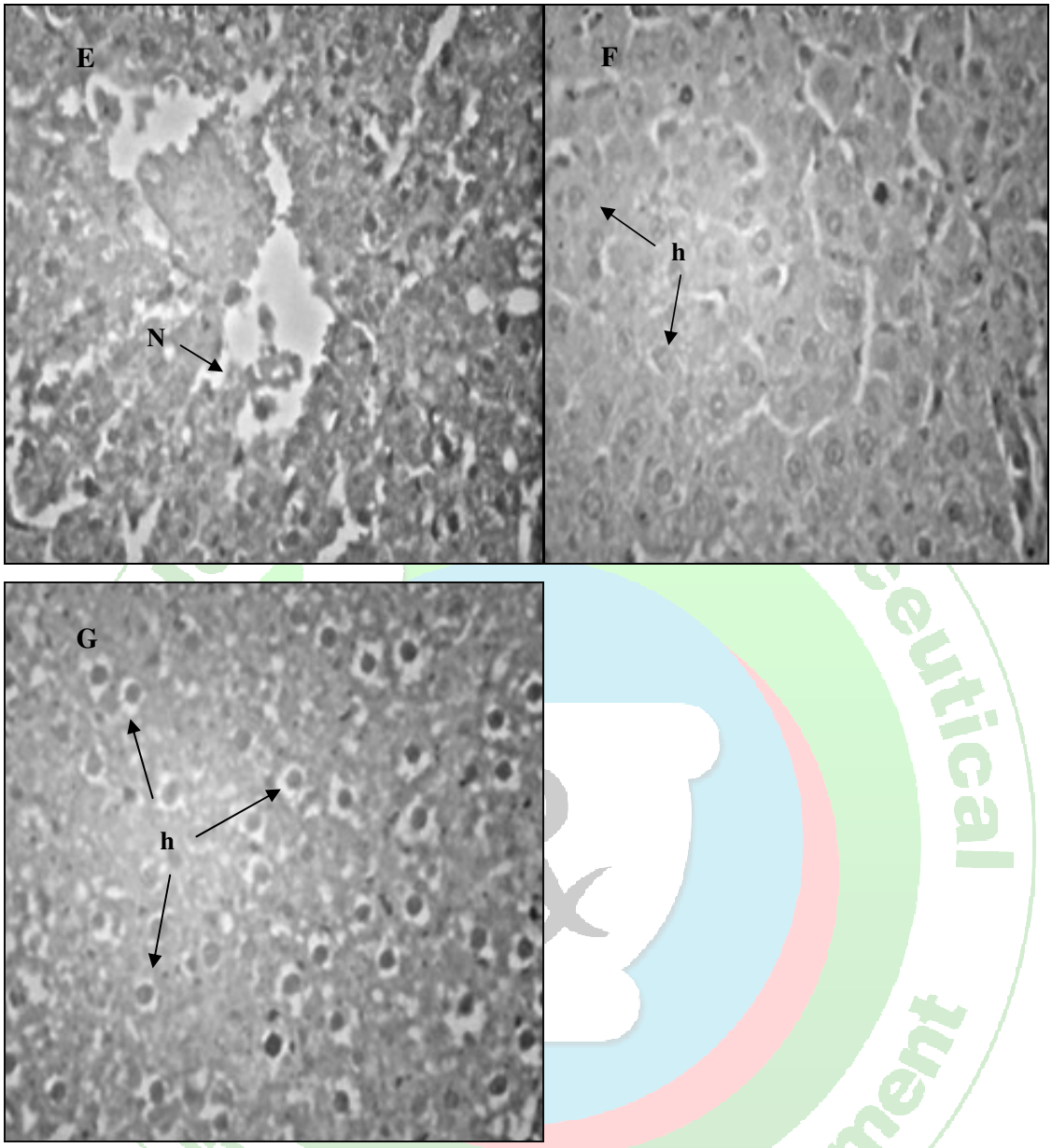


Fig.7: A-G represents the architecture of the hepatocytes in control, disease control, standard, fraction Ia, fraction Ib, fraction IIa and fraction IIb groups respectively

DISCUSSION

Preliminary phytochemical screening of *Aloe vera* gel extract showed the presence of carbohydrates and saponins. Existence of polysaccharide fractions were confirmed by analytical techniques, successfully isolated and were used for the study [12].

The drug was considered safe at doses 250 mg/kg and 500 mg/kg, *p.o.* as there was no mortality or morbidity and therefore these doses were selected as the final doses for the study [13-14].

Effect of Aloe vera fractions on the levels of SGPT

On treatment with single dose of paracetamol (3 g/kg, *p.o*), a marked release ($P< 0.001$) of SGPT was observed in disease control rats, indicating severe hepatic cell necrosis [15-16]. The levels of SGPT in the standard group were comparable to the control. Treatment with fraction Ia and Ib did not show any significant decrease in the levels of SGPT. Curative treatment with fraction IIb (500 mg/kg, *p.o*)

exhibited curative property almost equivalent to N-acetyl cysteine (450 mg/kg, *p.o)*, showing it's potential to maintain the normal functional status of the liver. Similar results have been reported by other investigators [17-18].

Effect of Aloe vera fractions on the levels of Total Bilirubin

Single dose of paracetamol intoxication caused significant increase ($P< 0.001$) in the levels of total bilirubin in disease control rats. The levels of total bilirubin in standard group were comparable with that of normal control. Curative treatment with fraction Ia, Ib and IIa did not show any siginificant decrease in the levels of total bilirubin i.e., *P* is not significant. Fraction IIb (500 mg/kg, *p.o*) exhibited curative action almost equivalent to N-acetyl cysteine 450 mg/kg, *p.o* i.e., *P* is highly significant ($P< 0.001$).

Effect of Aloe vera fractions on the levels of Direct Bilirubin

A significant increase ($P< 0.001$) in the levels of direct bilirubin was observed after intoxication of rats with single dose of paracetamol (3 g/kg, *p.o*) in disease control rats. The levels of direct bilirubin in standard group were comparable with that of normal control. Fraction Ia did not show any significant decrease in the levels of direct bilirubin. Fraction Ib ($P< 0.05$) and fraction IIa ($P< 0.01$) showed decrease in direct bilirubin levels in dose dependent manner. Fraction IIb showed significant ($P< 0.001$) restoration of bilirubin levels, showing it's curative potential which was almost equivalent to N-acetyl cysteine 450 mg/kg, *p.o.*

Effect of Aloe vera fractions on the levels of Serum Albumin

A significant decrease ($P < 0.001$) in the levels of serum albumin was observed with single dose of paracetamol intoxication (3 g/kg, *p.o*) in disease control rats. The curative potential of fraction Ia and Ib was not significant, which was evident from the *P*-value. Fraction IIa showed increase ($P< 0.01$) in the levels of serum albumin when compared to that of fractions Ia and Ib. Fraction IIb exhibited curative activity almost equivalent to N-acetyl cysteine 450 mg/kg, *p.o.* which was evident from the SEM values. Fraction IIb significantly ($P< 0.001$) restored the serum albumin levels to normal which showed the curative efficacy of the fraction.

Effect of Aloe vera fractions on the levels of GSH

A significant decrease ($P < 0.001$) in the levels of GSH was observed due to intoxication with single dose of paracetamol (3g/kg, *p.o*) in disease control rats. Paracetamol damage to liver significantly depleted the levels of GSH. NAPQI was mainly responsible for the depletion of GSH levels. Due to NAPQI formation following toxic paracetamol doses, GSH concentrations become very low in the centrilobular cells, which could account for the observed depletion in liver GSH stores [19]. Curative treatment with fraction Ia and Ib did not show any significant increase in the levels of GSH which was evident from the *P*-value. Curative treatment with fraction IIa increased the levels of GSH significantly ($P< 0.05$) when compared to that of fractions Ia and Ib. Curative treatment with fraction IIb increased the GSH values significantly ($P< 0.001$) when compared to the other fractions. The curative activity exhibited by fraction IIb was almost equivalent to the standard and similar results were reported by other investigators [20].

Effect of Aloe vera fractions on the levels of MDA

A significant increase ($P< 0.001$) in the levels of MDA was observed after intoxication of disease control rats with single dose of paracetamol (3 g/kg, *p.o*) due to the damage caused to cellular membrane by lipid peroxidation. Curative treatment with different doses (250 and 500 mg/kg) of *Aloe vera* fractions reversed the levels of MDA significantly towards the normal in a dose dependent manner. Treatment with fractions Ia and Ib did not show any significant decrease in the levels of MDA when compared to the normal. Fraction IIa showed significant decrease ($P< 0.05$) in the levels of MDA when

compared to the fractions Ia and Ib. Curative treatment with fraction IIb significantly ($P<$ 0.001) reversed the paracetamol intoxication induced increase in MDA and it exhibited curative action almost equivalent to N-acetyl cysteine 450 mg/kg, *p.o,* showing it's curative efficacy.

Mean and SEM values of all the parameters of various groups of animals were compared. The comparison gave the information regarding the efficacy of the fractions Ia, Ib, IIa and IIb. Mean and SEM values of IIb showed that the fraction was more effective in curing the liver insult caused by paracetamol, than fraction IIa.

Histopathology

Histopathology report of disease control rat confirmed the liver damage caused due to centrilobular necrosis. Normal hepatic architecture of control as well as standard group rats was confirmed by the histopathology study. Fraction Ia and Ib did not show any significant effect in restoring the integrity of hepatocytes and was confirmed by the histopathology report. Histopathology study confirmed the curative efficacy of fractions IIa and IIb against paracetamol induced liver damage as evident by the reversal of centrilobular necrosis in the surrounding hepatic parenchyma after administration of the fractions.

CONCLUSION

In conclusion, the possible mechanism of curative action of *Aloe vera* fraction IIb at a dose 500 mg/kg (*p.o.*) may be due to it's antioxidant activity as indicated by protection against increased lipid peroxidation and maintained glutathione contents. Rest of the biochemical parameters studied indicate the status of structural and functional integrity of the cells and provide further support to the suggestive mechanism of action. Since fraction IIb does not reveal any gross behavioral changes or mortality at the dose 500 mg/kg, p.o in rats and therefore it can be considered relatively safe. It can also be concluded that fraction II had more curative effect than fraction I and among fraction II, IIb was more effective than IIa.

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