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Original Research Article

Hepatoprotective activity of *Aloe vera* extract in a rodent model

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Abstract

In the present study, the preliminary phytochemical test was done on the alcoholic extract of *Aloe vera* leaf, and it was found to be rich in Carbohydrates, Proteins, saponins, Flavonoids, and Phenolic compounds. Plants have been used during the age for cure and treatment of diseases since the start of mankind. Phyto-therapy is the use of plants, plant extracts or pure chemicals isolated from natural products to treat diseases. Plants have been used to treat diseases such as diabetes, jaundice, cardiovascular disease, heavy metal poisoning, congestion of abdominal and pelvic cavities and scarlet fever etc. The plant has great potential in treating many ailments where the free radicals have been reported to be the major factors contributing to the disorders. In the present investigation, one of the traditionally used herbs, anti-hepatotoxicity being assessed against the Paracetamol-induced Hepatotoxicity in rats. Silymarin-treated animals also showed a significant ($p<0.001$) increase in antioxidant enzymes, namely SOD, catalase, GPx activities and GSH level compared to paracetamol-treated rats. To understand the effect of the extract on liver, histology of liver was performed.

Keywords: *Aloe vera*, Hepatotoxicity, Phytochemical Investigation, SOD, GSH.

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1. Introduction

Herbal medicine is the use of medicinal plants for the prevention and treatment of diseases, ranging from traditional and popular medicines found in every country to the use of standardized and titrated herbal extracts [1]. Generally cultural rootedness, enduring and widespread use in a Traditional Medical System may indicate safety, but not efficacy of treatments, especially in herbal medicine where tradition is almost completely based on remedies containing active principles at very low and ultra-low concentrations, or relying on magical-energetic principles [2-3].

In the age of globalization and of the so-called 'plate world', assessing the 'transferability' of treatments between different cultures is not a relevant goal for clinical research, while the assessment of efficacy and safety should be based on the regular patterns of mainstream clinical medicine [5-6]. The other black box of herbal-based treatments is the lack of definite and complete information about the composition of extracts [7].

Herbal-derived remedies require a comprehensive and in-depth assessment of their pharmacological properties and safety, which can be achieved through new biotechnological methods such as pharmacogenomics, metabolomics, and microarray methodologies [8]. Because of the large and growing use of natural-derived substances all over the world, it is not wise to rely also on tradition or supposed millenarian beliefs; explanatory and pragmatic studies are useful and should be considered complementary in the acquisition of reliable data both for health caregiver and patients [9-10].

Hepatotoxicity means damage to the liver caused by drugs and other factors, resulting in problems in its functioning. Chemicals or drugs that cause hepatotoxicity are called hepatotoxins [11-12]. The liver is the largest solid organ in the upper abdomen that aids in digestion and removes waste products and worn-out cells from the blood. It is considered to be one of the most vital organs that functions as the center of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites [13-15].

2. Materials and methods

2.1 Selection and collection of plant:

Plant and plant parts were selected based on the Ethno-botanical survey.

2.2 Authentication of the plant

The authentication of the plant was done by the botanist. A herbarium of plant was submitted to the specimen library of Safia College of Arts and Science, Peer Gate Bhopal, and authenticated by Dr. Ziaul Haque, Professor and head of the department of Botany, Safia College of Arts and Science, Peer Gate Bhopal. The specimen voucher no. of *Aloe vera* is 2024/201.

2.3 Extraction of plant material:

Aloe vera leaves are collected, washed with distilled water. Dried *Aloe vera* leaves were ground to powder form and stored in a tightly sealed container. The Soxhlet apparatus and method was used for extraction. The Soxhlet thimble was filled with the powdered leaves and inserted into the Soxhlet main chamber and closed [16]. One liter of 70% ethanol was filled into the Soxhlet main chamber and attached to the Soxhlet apparatus, which was heated until the solvent vapour filled the main chamber. The solvent vapour then condensed and dripped back down into the chamber containing the *Aloe vera* leaf extract. The *Aloe vera* leaf extract using 70% ethanol was then evaporated with a rotary evaporator at 30°C and concentrated to 50 mL before being freeze-dried. The powdered form of freeze-dried extract was kept in the freezer to maintain the compound [17].

2.4 Phytochemical investigation:

2.4.1 Qualitative photochemical investigation:

The phytochemical investigation was carried out by the procedure given in **Kokate *et al.*, 2006 [18]**.

***2.5 In-vivo* experiment:**

Table 1: Acute Toxicity Study (According to OECD 423)

IAEC Approval	All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (Reg No. 1824/PO/c/09/CPCSEA).
Animal used	*Albino wister* rat
Weight	150 to 200 gm
Age at the start of study	8-10 weeks
No. of animals/ dose Level	Three (03)
Acclimatization	One week prior to dosing
Identification of Animals	By cage number and marking on animal
Diet	Standard pellets (Golden Feeds, New Delhi)
Water	Purified water *ad libitum*
Sex	Male
Route of administration	P.O.
Housing Condition	Animals were housed in separate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (Golden Feed, New Delhi) and water regularly.

2.5.1 Methodology:

Acute oral toxicity (OECD 423)

The acute toxic class method set out in this Guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; no further testing is needed, dosing of three additional animals, with the same dose and, dosing of three additional animals at the next higher or the next lower dose level. 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 [19]**.**

2.5.2 Paracetamol induced hepatotoxicity:

A. Control group: served as control group treated with normal Saline at 1ml/kg body weight for 7 days

B. Vehicle treated group: served as Vehicle group treated with normal Saline at 1ml/kg body weight for 7 days.

C. 200 mg/kg extract treated group: Extract was dissolved in normal saline and was administered by oral route at a dose of 200m g/kg body weight each for 7 days

D. 400 mg/kg extract treated group: Extract were dissolved in normal saline and was administered by oral route at a dose of 400mg/kg body weight for 7 days.

On the seventh day, paracetamol suspension was given orally in a dose of 750 mg/kg to all except rats in group I, After 24 hours, all the rats were sacrificed under light ether anesthesia, and blood was collected in sterile Eppendorf tube and allowed to clot. Then the liver was isolated from the animal for *in-vivo* antioxidant assay and histology. Liver sections were homogenized by homogenizer for further studies**.**

***2.5.3 In vivo* oxidative stress**

In vivo Biochemical Assay for enzymes involved in oxidative stress

Preparing tissue for assay

Rinse organ with ice cold normal saline followed by 0.15 M tris HCl (pH 7.4) than performed as follow

1. GSH

10 % w/v in 0.1 M phosphate buffer (pH 7.4)

Glutathione (GSH)

Principle: DTNB is reduced in the presence of GSH to produce a yellow compound. The reduced chromogen is directly proportional to GSH conc. And its absorbance can be measured at 412 nm.

Conditions: T = 25□, pH = 7.0, A 412nm, Light Path = 1 cm

Method: Spectrophotometric estimation

Procedure

1. Prepare tissue homogenate (0.1 M phosphate buffer 7.4 pH , 10 %)
2. Take 0.2 ml of homogenate
3. Add 20 % TCA and 1 mM EDTA
4. Set aside for 5 min
5. Centrifuge 10 min at 2000 rpm
6. Take supernatant (200 µl) and transfer to new tube
7. Add 1.8 ml of Ellman's reagent (5,5'-dithio bis-2-nitrobenzoic acid (0.1mM) prepared in 0.3 M phosphate buffer, pH 7 with 1 % sodium citrate solution)
8. Make up volume upto 2 ml with distilled water
9. Take OD at 412 nm (water as blank)

Calculations

In Blood

= A sample x 66.66 mg/dL

= A sample x 2.22 mmol/dL

In Tissue

= (A sample x 66.66)/ g tissue used mg/ g tissue mg/g tissue

= (A sample x 2.22)/ g tissue used mmol/ g tissue mg/g tissue

2. SOD

The assay involves the production of superoxide from O_2 (using reduced bnicotinamide adenine dinucleotide (NADH) as a reductant, and phenazine methosulphate (PMS) as a catalyst) in the presence of an indicator, nitro blue tetrazolium (NBT), which turns blue when reduced by superoxide. The color change can be monitored spectro-photometrically in the visible range at 560 nm. When aliquots of common beverages are added to the reaction, superoxide scavengers (i.e., antioxidants) compete with NBT to react with superoxide. The percent inhibition of NBT reduction can be used to quantify superoxide-scavenging.

Conditions: T = 25□, pH = 7.0, A 560nm, Light Path = 1 cm

Method: Spectrophotometric determination

Procedure

1. Prepare 10 % w/v tissue homogenate in 0.15 M Tris HCl or, 0.1 M phosphate buffer
2. Centrifuge at 15000 rpm for 15 min at 4 °C
3. Take supernatant (0.1 ml), consider it as sample
4. 0.1 ml sample + 1.2 ml sodium pyrophosphate buffer (pH 8.3, 0.052 M) + 0.1 ml phenazine methosulphate (186 µM) + 0.3 ml of 300 µM Nitroblutetrazolium + 0.2 ml NADH (750 µM)
5. Incubate at 30°C for 90 s
6. Add o.1 ml glacial acetic acid
7. Stir with 4.0 ml n-butanol
8. Allow to stand for 10 min
9. Centrifuge and separate butanol layer
10. Take OD at 560 nm (take butanol as blank)

3. Results and discussion

3.1 Physical Examination:

Table 1: Physical Evaluation of *Aloe vera* leaf extract

S. No.	Organoleptic Characteristics	Result
1.	Colour	Dark Green
2.	Taste	Acrid
3.	Odour	Pungent
4.	Appearance	Semi-solid
5.	Consistency	Sticky

Solubility Tests:-

Table 2: Solubility of *Aloe vera* leaves extract in different solvents

S. No.	Solvent	Observation
1.	DMSO	Soluble
2.	Distilled water	Soluble
3.	Chloroform	Insoluble
4.	Methanol	Soluble

Table 3: Phytochemical Investigation

Test for carbohydrates	
Test Methanolic extract	
Molish	+Ve
Fehling's	+Ve
Benedict's	+Ve
Test for protein and amino acid	
Biuret	– Ve
Ninhydrin	– Ve
Test for glycosides	
Borntrager's	+ Ve
Keller-killani	+Ve
Test for alkaloids	
Mayer's	+Ve
Hager's	+ Ve
Wagner's	+ Ve
Test for saponins	
Froth Test + Ve	
Test for flavonoids	
Lead acetate	+ Ve
Alkaline reagent	+ Ve
Test for triterpenoids and steroids	
Salkowski's	+ Ve
Libermann-burchard's	+ Ve
Test for Tanin and phenolic compounds	
Ferric chloride	+Ve
Lead acetate	+ Ve
Gelatin	–Ve

Table 4: Acute Oral Toxicity

S. No.	Groups	Observations/ Mortality
1.	5 mg/kg	Bodyweight 0/3
2.	300 mg/kg Bodyweight	0/3
3.	2000 mg/kg Bodyweight	0/3

In Vivo Oxidative Stress Markers

Table 5: Effect of *Aloe vera* leaf extract on SOD in Paracetamol-induced oxidative stress in Liver.

S. No.	Groups	Absorbance
1.	**Control**	151.83±11.64
2.	**Vehicle**	56.47±31.53
3.	**Ex (200 mg/kg)**	78.36±28.92**
4.	**Ex (400 mg/kg)**	111.02±22.52**

Significantly different (P<0.05) as compared to the SOD level in the normal control group. Results are expressed as Mean±SD.

SOD and GSH enzymes are important scavengers. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage. In the present study, it was observed that the extract significantly increased the hepatic SOD activity in paracetamol-induced liver damage in rats. This show extract can reduce reactive free radicals that might lessen oxidative damage to the tissues and improve the activities of the hepatic antioxidant enzyme.

Table 6: Effect of *Aloe vera* leaf extract on GSH in Paracetamol-induced oxidative stress in Liver

S. No.	Groups	Absorbance
1.	Control	0.4352±0.75
2.	Vehicle	0.26± 0.066
3.	Ex (200 mg/kg)	0.32±0.052**
4.	Ex (400 mg/kg)	0.466±0.028**

The non-enzymic antioxidant, glutathione is one of the most abundant tripeptides present in the liver. Its functions are mainly concerned with the removal of free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals, and maintenance of membrane protein thiols and as a substrate for glutathione peroxidase and GST. In our present study Administration of extract 200mg/kg and 400 mg/kg significantly increased the level of glutathione in a dose-dependent manner.

In living systems, liver is considered to be highly sensitive to toxic agents. The study of different enzyme activities such as SGOT, SGPT, SALP have been found to be of great value in the assessment of clinical and experimental liver damage.

In the present investigation it was observed that the animals treated with paracetamol resulted in significant hepatic damage as shown by the elevated levels of serum markers. These changes in the marker levels will reflect in hepatic structural integrity. The rise in the SGOT is usually accompanied by an elevation in the levels of SGPT, which play a vital role in the conversion of amino acids to keto acids.

The treatment with extract at dose of 200mg/kg and 400mg/kg, significantly attenuated the elevated levels of the serum markers. The normalization of serum markers by extract suggests that they are able to condition the hepatocytes so as to protect the membrane integrity again paracetamol induced leakage of marker enzymes into the circulation.

The above changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells. Serum ALP levels, on the other hand are related to hepatic cell damage. Increase in serum level of ALP is due to increased synthesis in presence of increasing biliary pressure.

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