Research Article

Indian Journal of Science and Research

Thomas S. & Sharma VK., Ind. J. Sci. Res. 2022, 2(1), 16-21

ISSN 2583 – 2913

HEPATOPROTECTIVE ACTIVITY OF *ALOE VERA* EXTRACT IN RODENT MODEL

Hari Mohan*, Virendra Kumar Sharma and Steffi Thomas

Abstract*:* Present study was designed to evaluate Hepatoprotective Activity of *Aloe Vera* Extract in Rodent Model. The organoleptic characters of alcoholic extract of *Aloe vera* leaf extract were found to be dark green in colour, semi solid, and taste is acrid. alcoholic extract was observed to be dissolved in water and DMSO. Preliminary phyto-chemicals test was done on the alcoholic extract of *Aloe vera* leaf and it is found to be rich in Carbohydrates, Proteins, Saponin, Flavonoids and Phenolic compound. Acute oral toxicity of plant extract was performed to check the toxicity of plant and extract was found to be non toxic up to the dose of 2000 mg/kg. For the determination of protective effect of *Aloe vera* leaf extract against paracetamol induced Hepato-toxicity, firstly level of GSH and SOD was checked. GSH and SOD level was tested in vehicle treated group after that Paracetamol treated group and then 200mg/kg of plant extract along with Paracetamol treated group and after that 400mg/kg of plant extract along with Paracetamol treated group. In-vivo antioxidant activity was measured through a set of enzymes including SGOT, SGPT, and ALP. The extract administered at dose 400 mg/kg showed better activity.

Key-Words: *Aloe-vera*, Hepato-protective, Rodent Model, Paracetamol.

Introduction*:* Herbal medicine is the use of medicinal plants for prevention and treatment of diseases: it ranges from traditional and popular medicines of every country to the use of standardized and titrated herbal extracts. 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 [1].

Aloe vera has been found to have anti-inflammatory, wound healing, anti-diabetic, anticancer, antibacterial, antiviral, purgative, oxytocic and lipid lowering properties etc [2, 3, 4, 5]. Paracetamol (PCT) is a commonly used analgesic and antipyretic available over the counter. Liver is most susceptible to the toxic effects of PCT. The depletion of glutathione resulting in the accumulation of its toxic metabolite NAPQ1 causes hepatic dysfunction [6]. N-acetylcysteine and liver transplantation are the on available treatment options for paracetamol toxicity [7]. Hence there is a need for better treatment options for paracetamol induced hepato-toxicity. Some studies have demonstrated the hepatoprotective activity of *Aloe vera* against carbon tetrachloride and lindane induced hepato-toxicity in animal models. Hence the present study was undertaken to evaluate the hepatoprotective effect of *Aloe vera* in Paracetamol induced liver damage.

*Corresponding author

School of Pharmacy, LNCT University, Sarvadharm, C-Sector, Kolar Road Bhopal, Madhya Pradesh-462042, India.

E-Mail: hmohan634@gmail.com
Published on Web 30/04/2022, www.ijsronline.org

Materials and Methods

All the chemicals used in the study were procured from marck and Himedia. Plant and plant parts were selected on the basis of Ethano-botanical survey. The authentication of plant was done by the botanist. A herbarium of plants were submitted to the specimen library of safia college of arts and science, peer gate Bhopal.

Extraction of plant material: *Aloe vera* leaves 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. One liter of 70% ethanol was filled into the Soxhlet main chamber and attached to the Soxhlet apparatus, which was heated until the solvent vapor 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 o 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.

Phytochemical Investigation: The phytochemical investigation was carried out by using standard procedures [8].

In-vivo Study: Animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (Reg No. 1824/PO/c/09/CPCSEA).

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.

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 administrated 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 administrated by oral route at a dose of 400mg/kg body weight for 7 days.

On the seventh day, paracetamol suspension was given by oral 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 eppendroff tube and allowed to clot. Then liver were isolated from animal for *in-vivo* antioxidant assay and histology. Liver sections were homogenized by homogenizer for the further studies [9].

***In vivo* 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 presence of GSH to produce a yellow compound.

The reduced chromogen is directly propoertional to GSH conc. And its absorbance can be measured at 412 nm. [10]

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

Method: Spectrophotometric estimation

Procedure:

- Prepare tissue homogenate (0.1 M phosphate buffer 7.4 pH, 10 %)
- Take 0.2 ml of homogenate
- Add 20 % TCA and 1 mM EDTA
- Set aside for 5 min
- Centrifuge 10 min at 2000 rpm
- Take supernatant (200 µl) and transfer to new tube
- 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)
- Make up volume upto 2 ml with distilled water
- 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. Superoxide Dismutase (SOD): The assay involves the production of superoxide from O2 (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 spectrophotometrically 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.**[10]**

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

Method: Spectrophotometric determination

Procedure:

- Prepare 10 % w/v tissue homogenate in 0.15 M Tris HCl or, 0.1 M phosphate buffer
- Centrifuge at 15000 rpm for 15 min at 4 °C
- Take supernatant (0.1 ml), consider it as sample
- 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)
- Incubate at 30°C for 90 s
- Add o.1 ml glacial acetic acid
- Stir with 4.0 ml n-butanol
- Allow to stand for 10 min
- Centrifuge and separate butanol layer
- Take OD at 560 nm (take butanol as blank)

(1.5 unit/assay of the purified enzyme produced 80 % inhibition)

U/ml = % Inhibition x 3.75

U/gm tissue = % Inhibition x 3.75 x (1/gm tissue used)

3. Biochemical estimation: Blood samples were centrifuged for 10 min at 3000 rpm to separate the serum. AST, ALT, ALP, Total Bilirubin levels were estimated from the serum by using standard kits.

Results:

Physical Evaluation:

Table No. 1: Physical Evaluation of *Aloe vera* leaf extract

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

Solubility:

Table No. 2: Solubility of Aloe vera leaves extract in different solvents.

Sr. No	Solvent	Observation
1.	DMSO	Soluble
2.	Distilled water	Soluble
3.	Chloroform	InSoluble
4.	Methanol	Soluble

Phytochemical investigation:

Table No. 3: Phytochemical investigation of Aloe vera leaves extract

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

Acute Oral Toxicity:

Table No. 4: Acute toxicity studies of Aloe vera leaves extract

Sr. 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 No. 5: Effect of Aloe vera leaf extract on SOD in Paracetamol induced oxidative stress in Liver.

Sr. 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

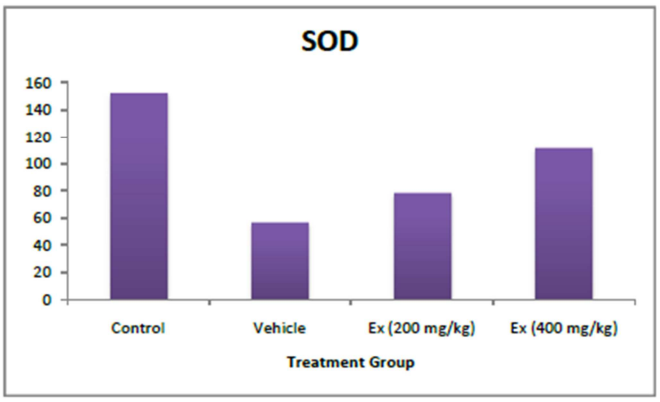

Figure 1: Effect of *Aloe vera* leaf extract on SOD in Paracetamol induced oxidative stress in Liver.

Table No. 6: Effect of *Aloe vera* leaf extract on GSH in Paracetamol induced oxidative stress in Liver

Sr. 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**

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

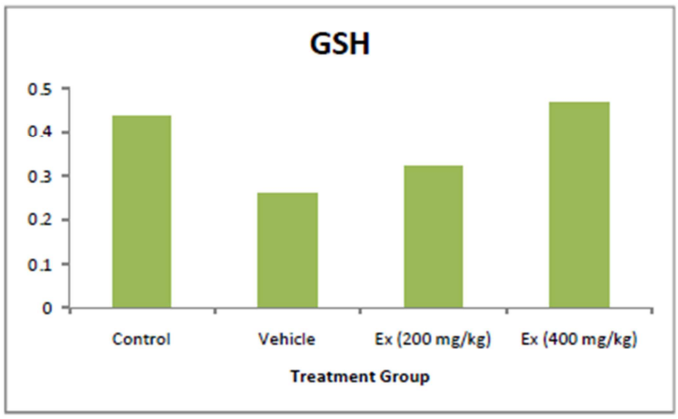

Figure 2: Effect of Aloe vera leaf extract on GSH in Paracetamol induced oxidative stress in Liver

Liver Function test:

Table No. 7: AST (SGOT), ALT (SGPT) and ALP

Sr. No	Groups	Absorbance SGOT	Absorbance ALT (SGPT)	Absorbance ALP
1.	**Control**	78.33±1.366	41.00±4.69	179.67±4.179
2.	**Vehicle**	221.33±10.875	162.83±8.424	306.83±4.708
3.	**Ex (200 mg/kg)**	271.83±9.087**	200.1±7.083**	105.5±2.429**
4.	**Ex (400 mg/kg)**	106.17±4.708**	77.00±3.795**	198.50±3.688**

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

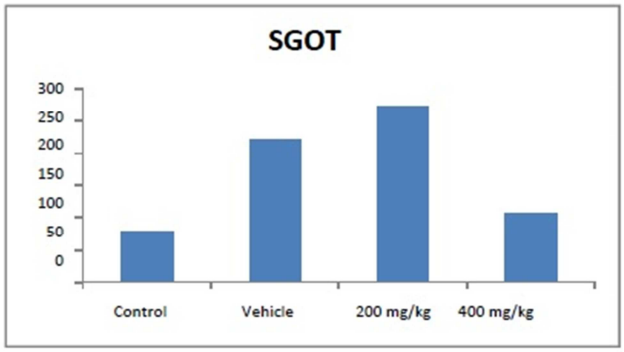

Figure 3: Effect of *Aloe vera* leaf extract on AST in Paracetamol induced Liver model.

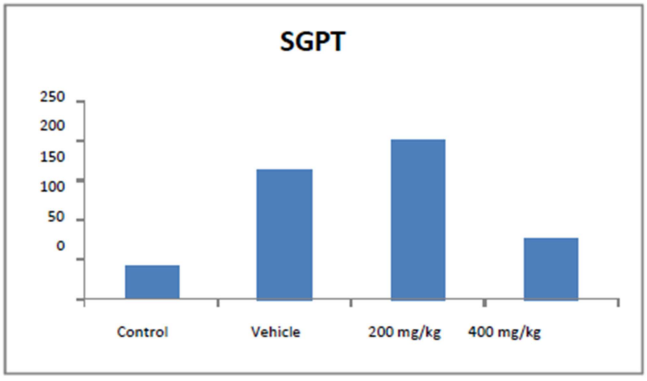

Figure 4: Effect of *Aloe vera* leaf extract on AlT in Paracetamol induced Liver Model.

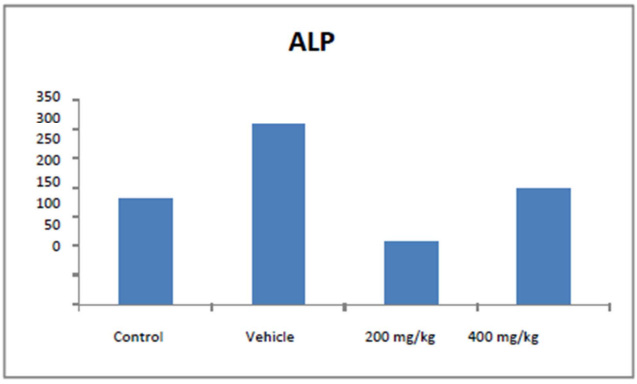

Figure 5: Effect of *Aloe vera* leaf extract on ALP in Paracetamol induced Liver model.

Discussion: Firstly, Organoleptic Characterization of plant extract was performed. Organoleptic evaluations are subjective, sensory judgments. They can involve eyeing, feeling and taste of the extract to the judge its appearance, coour, integrity, texture and flavours. The organoleptic characters of alcoholic extract of *Aloe vera* leaf extract were found to be dark green in colour, semi solid, and taste is acrid.

Solubility testing of alcoholic extract of *Aloe vera* leaf is done mainly to study the ability of the dissolve in different solvent for the preparation of aqueous extract for dosing. The alcoholic extract was observed to be dissolved in water and DMSO.

In the present study, the preliminary phytochemicals test was done on the alcoholic extract of *Aloe vera* leaf and it is found to be rich in Carbohydrates, Proteins, Saponin, Flavonoids and Phenolic compound.

In present study Acute oral toxicity of plant extract was performed to check the toxicity of plant and extract was found to be non toxic up to the dose of 2000 mg/kg. Hence, 2000mg/kg was considered as not observed adverse effect limit (NOAEL).1/10th and 1/5th of NOAEL, i.e. 200mg/kg and 400mg/kg were selected as dose for oral administration of extract.

For the determination of protective effect of *Aloe vera* leaf extract against paracetamol induced Hepato-toxicity, firstly level of GSH and SOD was checked. GSH and SOD level was tested in vehicle treated group after that Paracetamol treated group and then 200mg/kg of plant extract along with Paracetamol treated group and after that 400mg/kg of plant extract along with Paracetamol treated group.

In-vivo antioxidant activity was measured through a set of enzymes including SGOT, SGPT, and ALP. The levels were measured and it indicated that the extract had significant antioxidant activity however the results obtained were dose dependent the higher the dose (400 mg/kg) the better activity. The extract administered at dose 400 mg/kg showed better activity.

In animal treated with Paracetamol level of **SGOT** was 222.00±31.166, which were significantly higher ($P<0.001$) as compared to vehicle treated animals. The amino transferases are the most frequently utilized and specific indicators of hepatocellular necrosis.these enzymes Aspartate aminotransferase (AST, formerly serum glutamate oxaloacetic transaminase-SGOT) catalyze the transfer of the amino acids of aspartate and alanine respetivelyto the keto group of ketoglutaric acid.AST is present in a wide variety of tissues like the heart, skeletal muscle, kidney, brain and liver. Large increases in mitochondrial AST occur in serum after extensive tissue necrosis. Because of this, assay of

mitochondrial AST have been advocated in myocardial infarction. Mitochondrial AST is also increased in chronic liver diseases. In extract treated animals with 200mg/kg and 400mg/kg level of SGOT was found to be 106.25±4.349, 268.75±32.755 respectively which is significantly less (P<0.001) when compared with Paracetamol treated animals.

In animals treated with Paracetamol level of **SGPT** was 163.50±10.376, which were significantly higher (P<0.001) as compared to vehicle treated animals. The amino transferases are the most frequently utilized and specific indicator of hepatocellular necrosis.these enzymes Aspartate aminotransferase (AST, formerly serum glutamate oxaloacetic transaminase-SGOT) catalyze the transfer of the amino acids of aspartate and alanine respetivelyto the keto group of ketoglutaric acid. ALT is primarily localized to the liver. In extract treated group of animals with

200mg/kg and 400mg/kg level of SGPT was found to be 77.00±4.396, 199.50±11.818 respectively. The level of SGPT was significantly less (P<0.001) in extract treated (400mg/kg) group of animals.

Alkaline phophatases are a family of zinc metaloenzymes, with a serine at the active centre; they release inorganic phosphate from various organic orthophosphates and are in nearly all tissues. In liver, alkaline phosphatase is found histochemically in microvilli of the bile canaliculi and on the sinusoidal surface of hepatocytes. Highest level of alkaline phosphatase occur in cholestatic disorders. Elevation occurs as a result of both intraheptic and extraheptic obstruction to bile flow and the degree of elevation doeas not help to distinguish between the two. The mechanism by which alkaline phosphatase reaches the circulation is uncertain; leakage from the bile canaliculi into hepatic sinusoids may results from leaky tight junction and the other hypothesis is that the damaged liver fails to excrete alkaline phosphatase made in bone , intestine and liver. Level of ALP in 200mg/kg an 400mg/kg extract treated animal was 205.50±3.109, 106.25±4.349 respectively. It was significantly less (P<0.001) as compared to Paracetamol treated group.

Conclusion: Thus from present investigation it can be concluded that Aloevera leaf extract possess significant protective potential against Paracetamol induced Hepatotoxicity. Being one of the easily available trees it can be a good source of components with hepatoprotective agent.

References:

1. Firenzuoli, F., & Gori, L. (2007). Herbal medicine today: clinical and research issues. *Evidence-Based Complementary and Alternative Medicine*, *4*(S1), 37-40.
2. Reynolds T, Dweck AC. Aloe vera leaf gel; a review update. J Ethanopharmacol 1999; 68:3- 37.
3. Chandan BK, Saxena AK, Shukla S ,et al. Hepatoprotective potential of Aloe barbadensis Mill against carbon tetrachloride induced hepatotoxicity. J Ethanopharmacolgy 2007; 111(3):560-66.
4. Etim OE, Farombi EO, Usoh IF, Akpan EJ. The protective effect of aloe vera juice on lindane induced hepatotoxicity and genotoxicity. Pak J Pharm Sci 2006; 19(4):333-37.
5. Kim SH, Cheon HJ, Yun N, et al. Protective effect of a mixture of aloe vera and silybum marianum against carbon tetrachloride induced acute hepatotoxicity and liver fibrosis. J Pharmacol Sci 2009;109: 119-27.
6. O'Grandy JG. Paracetamol hepatotoxicity how to prevent. J Royal soci medi .1997; 90:368-70.
7. Vitols S. Paracetamol hepatotoxicity at therapeutic doses. J Inter Med 2003; 253:95-98
8. Kokate C.K., Purohit A.P., and Gokhale S.B. (2006) Pharmacognosy; 23 ed., Nirali prakashan: pp:- 493-497.
9. Simon rama parmar , patel hitesh vashrambhai , kiran kalia. Hepatoprotective activity of some plants extract against paracetamol induced hepatotoxicity in rat journal of herbal medicine and toxicology 4 (2) 101-106 (2010).
10. Amira A. Shaheen, Amal A. Abd El-Fattah, Effect of dietary zinc on lipid peroxidation, glutathione, protein thiols levels and superoxide dismutase activity in rat tissues, The International Journal of Biochemistry & Cell Biology, Volume 27, Issue 1, 1995, Pages 89-95.