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STUDIES ON ASSESSMENT OF ENZYMATIC ANTIOXIDANT POTENTIALS OF *ALOE VERA* L. LEAF EXTRACTS ON OXIDATIVE STRESS INDUCED *IN VITRO* ANIMAL MODEL

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ABSTRACT

Traditional plant-based remedies are finding increasing application as a source of direct therapeutic agent. Presently, plants are the original source materials for as many as 40% of pharmaceuticals currently used around the world. These drugs either contain plant-derived materials, or materials synthesized from agents originally derived from plants. Innumerable therapeutic agents can be obtained from medicinal plants after screening of secondary metabolite compounds. Since ancient time *Aloe vera* L. are using in many herbal preparations as antioxidants. *Aloe vera* L. possesses an important role in absorbing and neutralizing oxidant induced free radicals. In present investigation the enzymatic antioxidant activities of *in vivo* grown and *in vitro* propagated *Aloe vera* L. whole leaves and only gel extracts against oxidative stress induced *in vitro* animal model were examined. Here in current study, we used *in vitro* animal models as an alternate of using live animals in laboratory to reduce the harm and pain to live animals during laboratory experiments. Results were showing that among the different samples of methanol extracts of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaves and inner gel, the methanol extracts with *in vitro* regenerated *Aloe vera* L. leaves were showing higher degree of protection against oxidant induced stress in *in vitro* animal model.

KEYWORDS: *Aloe vera* L., *in vitro* animal models, oxidant induced stress, oxidative stress, free radicals, reactive oxygen species and enzymatic antioxidants.

INTRODUCTION

Plant-derived medicines are the most widely used medicines in the world today. The use of herbs and plants as first medication is practiced universally. Every culture on earth, through written or oral tradition, has relied on the vast variety of natural chemistry found in healing plants for their therapeutic properties. Plants with therapeutic potential may be defined as any plant that can be put to culinary and/or medicinal use. Recent researchers have found that food and their constituents act in a manner similar to modern drugs without dreaded side effects.[1]

Reactive oxygen species (ROS) are normal by-products of cellular metabolism.[2] Reactive oxygen species are unstable and aggressive molecules, which have the tendency to give their unpaired electron to other cellular molecules or snatch electrons from other molecules to attain stability.[3] The production of reactive oxygen species may produce oxidative stress and induce various degenerative diseases.[4]

Oxidative stress occurs when the production of reactive oxygen species overrides the antioxidant capacity in the target cell, resulting in the damage of macromolecules such as lipids, nucleic acids and proteins, causing alterations in the target cell function and leading to cell death.[5]

The role of free radicals and tissue damage in diseases, such as atherosclerosis, heart failure, neurodegenerative disorders, aging, cancer, diabetes mellitus, hypertension and several other diseases, is gaining a lot of recognition.[6] Both reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are products of normal cellular metabolism. They are well recognized as

playing a dual role as both deleterious and beneficial species, in that they can be either harmful or beneficial to living systems.

Antioxidant supplements, or foods rich in medicinal plants, may be used to help the human body in reducing oxidative damage by free radicals and active oxygen.[7]

In the human and animal body, ROS can be neutralized by antioxidant defence systems including antioxidant enzymes and antioxidant compounds.[8]

Currently, research interest has been focused on the role of antioxidants as well as antioxidant enzymes, in the treatment and prevention of the diseases mentioned above. The most commonly used antioxidants at present are vitamins, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and *tert*-butylhydroquinone (TBHQ). However, they are suspected of being responsible for liver damage and acting as carcinogens in laboratory animals.[9] Therefore, the development and utilization of more effective antioxidants of natural origin is desirable.

Alternatives of live experimental animals (*in vitro* models) to examine the antioxidant activities or protective effects in laboratory

Various *in vitro* model organisms have been used for scientific research purposes.[10,11]

In vitro model organisms are used in biomedical research for the following reasons

- To overcome ethical and experimental issues,
- To develop a tool to optimize methods and protocols to facilitate and standardize analysis,
- It represents a major class of living beings with complex biological systems.[12]

Worldwide, scientific research uses millions of animals in experiments every year. The main thrust of the ethical argument against animal experiments is that animals, at least vertebrates, can experience pain and suffering. Apart from that, they can be harmed by confinement, frustration, fear, isolation and loss of life.[13] Non-animal approaches are now considered as advanced methods, which can overcome many of the limitations of animal experiments. Replacing animal procedures with methods such as cells and tissues *in vitro*, volunteer studies, physicochemical techniques and computer modelling, is driven by legislative, scientific and moral imperatives.[14]

Precision-cut tissue slices represent an organ mini-model that closely resembles the organ from which it is prepared, with all cell types present in their original tissue matrix configuration.[15] Organ slices, an *in vitro* model representing the multicellular, structural and functional features of *in vivo* tissue, is a promising model for elucidating mechanisms of drug-induced organ injury and for characterizing species susceptibilities. The liver is the major organ used in organ slice studies.[16]

There is an increasing interest in developing alternatives to animal testing and the three R's of Reduction, Refinement and Replacement (Reduction of numbers of animals used, Refinement of housing, handling and experimental procedures to reduce discomfort, pain, fear, stress and suffering and Replacement of experimental animals by alternatives) are the basis due to a variety of reasons, including impeding regulatory initiatives, new assessments of the performance of the accepted battery of *in vitro* tests and new knowledge in cancer biology.[17]

In the present study, precision cut goat liver slices were used as an *in vitro* model (alternatives to the live animals). The liver slices were subjected to oxidative stress and effect of the methanol extracts of *Aloe vera* L. whole leaves and inner gel (including *in vivo* and *in vitro* regenerated *Aloe vera* L.) in counteracting this stress was assessed by analysing the antioxidant status.

MATERIALS AND METHODS

Preparation of crude extract

Leaves of the *Aloe vera* L. were collected from the already *in vitro* propagated and properly acclimatized 9-12 months old plants. *In vitro* propagation was the previous phase of our study to produce quality plant material to meet industrial requirement. Simultaneously leaves from 9-12 months old *in vivo* grown *Aloe vera* L. plants were also collected. Freshly collected *Aloe vera* L. leaves were washed with distilled water, followed by disinfecting with ethanol 70%. Later, in case of whole leaf crude extract preparation, leaves were chopped into the small pieces and were exposed to 50ºC for 3 days to get dried. After complete drying, leaf parts were powdered using electric grinder, simultaneously in case of only gel crude extract preparation, upper green skin/rind of leaves was removed and latex was cut into small pieces and both types of leaf materials were homogenized separately. The homogenized materials were extracted with ethanol (95%). The ethanol from the extracted leaf materials was evaporated at 65^{o}C temperature in water bath. The solvent was completely removed and dried to get powder. All the powdered plant materials including whole leaf and only gel were used for the preparation of solvent extracts.

Preparation of solvent extracts

Extracts were prepared using the modified method of Case[18]. 1:3 (w/v) ratios were used for the powdered leaf material and methanol as solvent for extract preparation. The pulverized leaves material was mixed with sufficient quantity of solvent i.e. methanol and then it was kept in rotary shaker at 100 rpm overnight and filtered with Whatman No.1 filter paper and subsequently subjected to lyophilization at – 47.5ºC. The dried extracts thus obtained was weighed, transferred into separate vials and preserved at 4ºC for future analysis.

The goat liver was collected fresh from a slaughter house, plunged into cold sterile phosphate buffered saline (PBS) and maintained at 4°C till use. Thin slices

of 1mm thickness were cut using a sterile scalpel. One gram of goat liver slice was taken in 4.0ml of sterile PBS in a broad flatbottomed vessel. The oxidant used was H_2O_2 at a final concentration of 500µM. The oxidizing agent H_2O_2 (500µM) and/or the *Aloe vera* L. leaf extracts (500mg/kg tissue) were added and the slices exposed to different treatments were incubated for one hour at 37°C with mild shaking. Appropriate controls were also set up. After incubation, a homogenate was prepared in phosphate buffered saline, held on ice and the antioxidant status was analysed. For each parameter, the incubation of tissue slices with oxidant and plant extracts was carried out separately and fresh on the day of the experiment.

Enzymatic antioxidant status in goat liver slices (*in vitro* animal model) exposed to oxidative stress

The enzymatic antioxidants that were analysed were superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione s-transferase (GST) and glutathione reductase. Liver homogenate was used as enzyme source for the assays.

Assay of superoxide dismutase (SOD)

SOD was assayed according to the method of Kakkar *et al.*[19] The assay of SOD is based on the inhibition of the formation of NADH-phenazine methosulphate-nitroblue tetrazolium formazon. The colour formed at the end of the reaction can be extracted into butanol and measured at 560nm. The reagents used were Sodium pyrophosphate buffer (0.025M, pH 8.3), Phenazine methosulphate (PMS) (186µM), Nitroblue tetrazolium (NBT) (300µM), NADH (780µM), Glacial acetic acid, n-butanol and Potassium phosphate buffer (50mM, pH 6.4).

Preparation of enzyme extract

Samples from *Aloe vera* L. leaf crued extract (0.5g) were taken and mixed with 3.0ml of potassium phosphate buffer, centrifuged at 2000g for 10 minutes and the supernatants were used for the assay.

Assay

The assay mixture contained 1.2ml of sodium pyrophosphate buffer, 0.1ml of PMS, 0.3ml of NBT, 0.2ml of the enzyme preparation and water in a total volume of 2.8ml. The reaction was initiated by the addition of 0.2ml of NADH. The mixture was incubated at 30°C for 90 seconds and arrested by the addition of 1.0ml of glacial acetic acid. The reaction mixture was then shaken with 4.0ml of n-butanol, allowed to stand for 10 minutes and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560nm in a spectrophotometer. One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in one minute.

Assay of catalase (CAT)

Catalase activity was assayed following the method of Luck.[20] The UV absorption of hydrogen peroxide can be measured at 240nm, whose absorbance decreases when degraded by the enzyme catalase. From the decrease in absorbance, the enzyme activity can be calculated. Reagents used were Phosphate buffer: 0.067 M (pH 7.0) and Hydrogen peroxide (2mM) in phosphate buffer.

Preparation of enzyme extract

A 20% homogenate of *Aloe vera* L. leaf was prepared in phosphate buffer. The homogenate was centrifuged and the supernatant was used for the enzyme assay.

Assay

H_2O_2-phosphate buffer (3.0ml) was taken in an experimental cuvette, followed by the rapid addition of 40µl of enzyme extract and mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240 nm in a spectrophotometer. The enzyme solution containing H_2O_2-free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240nm by 0.05 units.

Assay of peroxidase (POD)

The method proposed by Reddy *et al.*[21] was adopted for assaying the activity of peroxidase. In the presence of the hydrogen donor pyrogallol or dianisidine, peroxidise converts H_2O_2 to H_2O and O_2. The oxidation of pyrogallol or dianisidine to a coloured product called purpurogalli can be followed spectrophotometrically at 430nm. The reagents used were Pyrogallol: 0.05 M in 0.1M phosphate buffer (pH 6.5) and H_2O_2 : 1% in 0.1M phosphate buffer, pH 6.5.

Preparation of enzyme extract

A 20% homogenate was prepared in 0.1M phosphate buffer (pH 6.5) from the leaf of the plant, clarified by centrifugation and the supernatant was used for the assay.

Assay

To 3.0ml of pyrogallol solution, 0.1ml of the enzyme extract was added and the spectrophotometer was adjusted to read zero at 430 nm. To the test cuvette, 0.5ml of H_2O_2 was added and mixed. The change in absorbance was recorded every 30 seconds up to 3 minutes in a spectrophotometer. One unit of peroxidase is defined as the change in absorbance/minute at 430nm.

Assay of glutathione s-transferase (GST)

Glutathione S-transferase was assessed by the method of Habig *et al.*[22] The enzyme is assayed by its ability to conjugate GSH and CDNB, the extent of conjugation causing a proportionate change in the absorbance at 340nm. The reagents used were Glutathione (1mM), 1-chloro-2,4-dinitrobenzene (CDNB) (1mM in ethanol) and Phosphate buffer (0.1M, pH 6.5).

Preparation of enzyme extract

The samples (0.5g) were homogenized with 5.0ml of phosphate buffer. The homogenates were centrifuged at 5000rpm for 10 minutes and the supernatants were used for the assay.

Assay

The activity of the enzyme was determined by observing the change in absorbance at 340nm. The reaction mixture contained 0.1ml of GSH, 0.1ml of CDNB and phosphate buffer in a total volume of 2.9ml. The reaction was initiated by the addition of 0.1ml of the enzyme extract. The readings were recorded every 15 seconds at 340nm against distilled water blank for a minimum of three minutes in a spectrophotometer. The assay mixture without the extract served as the control to monitor nonspecific binding of the substrates. GST activity was calculated using the extinction co-efficient of the product formed ($9.6mM^{-1}cm^{-1}$) and was expressed as nmoles of CDNB conjugated/minute.

Assay of glutathione reductase

Glutathione reductase was assayed by the procedure adopted by David and Richard.[23] Glutathione reductase catalyses the conversion of oxidized glutathione to reduced glutathione employing NADPH as substrate. The amount of NADPH utilized is a direct measure of enzyme activity. The reagents used were Phosphate buffer (0.12M, pH 7.2), EDTA (15mM), Sodium azide (10mM), Oxidized glutathione (6.3mM) and NADPH (9.6mM).

Assay

The assay system contained 1ml of phosphate buffer, 0.1ml of EDTA, 0.1ml of sodium azide, 0.1ml of oxidized glutathione and 0.1ml of enzyme source and the volume was made up to 2ml with distilled water. The tubes were incubated for 3 minutes and 0.1ml of NADPH was added. The absorbance was read at 340nm in a spectrophotometer at every 15 seconds interval for 2-3 minutes. For each series of measurement, controls were set up that contained water instead of oxidized glutathione. The enzyme activity was expressed as µmoles of NADPH oxidized/minute/g liver tissue.

Statistical analysis

All the analysis were carried out in triplicates and expressed as mean ± SD. Analysis of variance (ANOVA) were performed using the one-way analysis of variance. Significant differences between means were determined by Duncan's multiple range tests. P values less than 0.05 were considered statistically significant.

RESULTS

In present investigation the antioxidant effects of methanol extracts with all the samples of whole leaf and inner gel taken from *in vivo* as well as *in vitro* regenerated *Aloe vera* L. were examined using goat liver slices as an *in vitro* model which mimics the *in vivo* conditions to avoid the use of live animals and less harm them in laboratory experiments.

Enzymatic antioxidant activities of *Aloe vera* L. on oxidant induced damage in the *in vitro* model (goat liver slices) analyzed were SOD, CAT, POD, GST and GR.

Results obtained are shown in the Table 1 – 5. The exposure of liver slices to H_2O_2 caused a steep decrease in the activities of all the enzymatic antioxidants analyzed. The co-treatment with the methanol extract of *Aloe vera* L. leaf and inner gel significantly ($p < 0.05$) improved the enzymatic antioxidant status in the oxidant induced stressed goat liver slices. Sample V containing methanol extract of *in vitro* regenerated *Aloe vera* L. inner gel was showing the higher enzymatic antioxidant activity, regarding all the enzymatic antioxidants tested including SOD, CAT, POD, GST and GR. Therefore, co-treatment of the sample was significantly effective compared to H_2O_2 alone treated goat liver slices. While sample IV, III and II were also showing the significant increase in the enzymatic antioxidant levels when co-treated, compared to H_2O_2 alone treated goat liver slices (Table 1 – 5).

Table 1: Effect of *Aloe vera* L. leaf extract on SOD activity in goat liver slices exposed to H_2O_2 *in vitro*

S.No.	Sample	SOD activity (Units*/g tissue)	
		Without H_2O_2	With H_2O_2
1	Sample I	10.36 ± 1.42	6.57 ± 0.38[a]
2	Sample II	12.82 ± 0.60[a]	9.26 ± 0.53[abc]
3	Sample III	14.39 ± 0.24[a]	12.83 ± 0.16[abc]
4	Sample IV	15.24 ± 0.32[a]	14.33 ± 0.37[abc]
5	Sample V	18.47 ± 0.84[a]	16.29 ± 0.51[abc]

The values are mean ± SD of triplicates.
*1 Unit = Amount of enzyme that caused 50% reduction in NBT oxidation in one minute.
a- Statistically significant (P<0.05) compared to untreated control
b- Statistically significant (P<0.05) compared to H_2O_2 alone treated group
c- Statistically significant (P<0.05) compared to the respective extract treated group

Sample I – No extract
Sample II – Methanol extract of *in vivo* grown *Aloe vera* L. whole leaf samples
Sample III – Methanol extract of *in vivo* grown *Aloe vera* L. only gel samples
Sample IV – Methanol extract of *in vitro* regenerated *Aloe vera* L. whole leaf samples
Sample V – Methanol extract of *in vitro* regenerated *Aloe vera* L. only gel samples

Table 2: Effect of *Aloe vera* L. leaf extract on CAT activity in goat liver slices exposed to H_2O_2 *in vitro*.

S.No.	Sample	CAT activity (Units[&]/g tissue)	
		Without H_2O_2	With H_2O_2
1	Sample I	138.21 ± 12.39	92.73 ± 4.04[a]
2	Sample II	153.39 ± 14.71[a]	108.27 ± 5.18[abc]
3	Sample III	176.05 ± 14.98[a]	114.16 ± 6.62[abc]
4	Sample IV	197.34 ± 15.37[a]	127.84 ± 9.28[abc]
5	Sample V	223.80 ± 18.22[a]	142.52 ± 11.36[abc]

The values are mean ± SD of triplicates.
&1 Unit = Amount of enzyme required to decrease the absorbance at 240nm by 0.05 units/minute.
a- Statistically significant (P<0.05) compared to untreated control
b- Statistically significant (P<0.05) compared to H_2O_2 alone treated group
c- Statistically significant (P<0.05) compared to the respective extract treated group

Sample I – No extract
Sample II – Methanol extract of *in vivo* grown *Aloe vera* L. whole leaf samples
Sample III – Methanol extract of *in vivo* grown *Aloe vera* L. only gel samples
Sample IV – Methanol extract of *in vitro* regenerated *Aloe vera* L. whole leaf samples
Sample V – Methanol extract of *in vitro* regenerated *Aloe vera* L. only gel samples

Table 3: Effect of *Aloe vera* L. leaf extract on POD activity in goat liver slices exposed to H_2O_2 *in vitro*.

S.No.	Sample	POD activity (Units[$]/g tissue)	
		Without H_2O_2	With H_2O_2
1	Sample I	31.95 ± 0.16	24.16 ± 0.51[a]
2	Sample II	36.24 ± 0.28[a]	29.71 ± 0.58[abc]
3	Sample III	42.37 ± 0.32[a]	36.24 ± 0.60[abc]
4	Sample IV	48.23 ± 0.38[a]	43.05 ± 0.63[abc]
5	Sample V	53.82 ± 0.41[a]	49.28 ± 0.74[abc]

The values are mean ± SD of triplicates.
$1 Unit = Change in absorbance at 430nm per minute.
a- Statistically significant (P<0.05) compared to untreated control
b- Statistically significant (P<0.05) compared to H_2O_2 alone treated group
c- Statistically significant (P<0.05) compared to the respective extract treated group

Sample I – No extract
Sample II – Methanol extract of *in vivo* grown *Aloe vera* L. whole leaf samples
Sample III – Methanol extract of *in vivo* grown *Aloe vera* L. only gel samples
Sample IV – Methanol extract of *in vitro* regenerated *Aloe vera* L. whole leaf samples
Sample V – Methanol extract of *in vitro* regenerated *Aloe vera* L. only gel samples

Table 4: Effect of *Aloe vera* L. leaf extract on GST activity in goat liver slices exposed to H_2O_2 *in vitro*.

S.No.	Sample	GST activity (Units[@]/g tissue)	
		Without H_2O_2	With H_2O_2
1	Sample I	1.02 ± 0.03	0.83 ± 0.004[a]
2	Sample II	1.18 ± 0.06[a]	0.98 ± 0.02[abc]
3	Sample III	1.23 ± 0.08[a]	1.01 ± 0.02[bc]
4	Sample IV	1.26 ± 0.10[a]	1.05 ± 0.02[bc]
5	Sample V	1.28 ± 0.14[a]	1.12 ± 0.06[abc]

The values are mean ± SD of triplicates.
@1 Unit = nmoles of CDNB conjugated per minute.
a- Statistically significant (P<0.05) compared to untreated control
b- Statistically significant (P<0.05) compared to H_2O_2 alone treated group
c- Statistically significant (P<0.05) compared to the respective extract treated group

Sample I – No extract
Sample II – Methanol extract of *in vivo* grown *Aloe vera* L. whole leaf samples
Sample III – Methanol extract of *in vivo* grown *Aloe vera* L. only gel samples
Sample IV – Methanol extract of *in vitro* regenerated *Aloe vera* L. whole leaf samples
Sample V – Methanol extract of *in vitro* regenerated *Aloe vera* L. only gel samples

Table 5: Effect of *Aloe vera* L. leaf extract on GR activity in goat liver slices exposed to H_2O_2 *in vitro*.

The values are mean ± SD of triplicates.

S.No.	Sample	GR activity (Units[#]/g tissue)	
		Without H_2O_2	With H_2O_2
1	Sample I	5.04 ± 0.14	2.13 ± 0.72[a]
2	Sample II	6.39 ± 0.36[a]	4.14 ± 0.06[abc]
3	Sample III	7.28 ± 0.33[a]	6.48 ± 0.04[abc]
4	Sample IV	8.36 ± 0.20[a]	7.02 ± 0.08[abc]
5	Sample V	9.47 ± 0.41[a]	8.26 ± 0.07[abc]

#1 Unit is defined as the milli moles of NADPH oxidized / minute
a- Statistically significant (P<0.05) compared to untreated control
b- Statistically significant (P<0.05) compared to H_2O_2 alone treated group
c- Statistically significant (P<0.05) compared to the respective extract treated group

Sample I – No extract
Sample II – Methanol extract of *in vivo* grown *Aloe vera* L. whole leaf samples

Sample III – Methanol extract of *in vivo* grown *Aloe vera* L. only gel samples
Sample IV – Methanol extract of *in vitro* regenerated *Aloe vera* L. whole leaf samples
Sample V – Methanol extract of *in vitro* regenerated *Aloe vera* L. only gel samples

DISCUSSION

In this study, the enzymatic antioxidant potential of methanol extract of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaves and inner gel was estimated in an *in vitro* model stimulating the *in vivo* system, namely goat liver slices. Organ slices, an *in vitro* model representing the multicellular, structural and functional features of *in vivo* tissue, is a promising model for elucidating mechanism of drug induced-organ injury and for characterizing species susceptibilities. The liver is the major organ used in organ slice studies.[24]

Liver, the largest organ in vertebrate body, has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver. Even common dietary antioxidants can provide such protection from liver damage caused by oxidative mechanisms of toxic chemicals. A phytotherapeutic approach to modern drug development can provide many invaluable drugs from traditional medicinal plants such as *Aloe vera* L.[25]

Liver slices are an intermediate between liver cells and isolated organs. A major advantage of hepatic slices compared to isolated hepatocytes is the lack of disruption of cell-to-cell contacts as occurring during the hepatocyte isolation procedure. With liver slices, the normal tissue architecture, cell heterogeneity and cell-cell interactions are maintained; the native cell types and integrity of the organ remains intact.[26]

Therefore, during the last two decades, substantial efforts have been made towards the development and international acceptance of alternative methods to safety studies using laboratory animals. Animal testing should cause as little suffering to animals as possible, and those animal tests should only be performed where necessary.

Hence, in order to minimize the use of live animals, this study has been carried out using alternative model system, namely goat liver tissue. As precision-cut liver slices are an appropriate model of *in vitro* systems for many reasons, including simplicity and ease of preparation, retention of normal organ architecture, and the ability to obtain multiple slices from each organ.[27]

Thin slices of liver tissue exposed to the oxidant (H_2O_2) in the presence and the absence of the extract were used. The protective effects of the methanol extract of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and inner gel, *in vitro* against hydrogen peroxide-induced oxidative stress were evaluated and the enzymatic antioxidants were analysed in the slices.

The enzymatic antioxidants analysed were SOD, CAT, POD, GST and GR.

Superoxide dismutase (SOD)

Superoxide dismutase is the primary defence chain breaking antioxidant which catalyses the conversion of highly reactive superoxide anion radical produced during electron transfer reactions in mitochondria to less reactive hydrogen peroxide and prevents lipid peroxidation.[28,29] The exposure of liver slices to hydrogen peroxide significantly decreased the activity of SOD compared to the untreated control. The treatment with the methanol extract of whole leaf and inner gel of *in vitro* and *in vivo* grown *Aloe vera* L., increased the SOD activity significantly compared to the untreated control. The co-administration of either of the extracts along with H_2O_2 showed an increase in SOD activity compared to H_2O_2 alone treated group.

An increased level of SOD was also noticed in a glycoportein isolated from *Rhus verniciflua* in the liver of hyperlipidemic mice.[30] The green pods of *Acacia nilotica* caused a significant increase in the levels of SOD in the liver, lungs, kidneys and blood in CCl_4 treated rats.[31] The administration of methanolic extract of the *Lysmachia clethroides* Duby to the CCl_4 induced Kunming mice increased the SOD activity in mice liver.[32]

The administration of methanolic extract of *Decalpis hamiltonii* roots to injured rats (induced by acetaminophen) increased the activity of enzymic antioxidants SOD and CAT in the liver of rats.[33] The treatment with the alkaloid caffeine also increased the activities of enzymatic antioxidants SOD in the liver of male Wistar rats.[34]

The results from our current investigations, supported by the above quoted literature, also showed that the methanol extract of whole leaf and inner gel of *in-vitro* and *in-vivo* grown *Aloe vera* L., have the ability to increase the superoxide activity during oxidatively stressed conditions suggesting their antioxidant influencing activity.

Catalase (CAT)

Catalase is the second major defensive antioxidant enzyme next to superoxide dismutase. It catalyses the conversion of hydrogen peroxide to water and molecular oxygen.[35] and prevents the formation of hydroxyl radical.[36]

In the present study, the activity of catalase was found to decrease on exposure of the slices to the oxidant hydrogen peroxide. This may be due to the utilization of

the enzyme in the detoxification of hydrogen peroxide. However, on administration of the extract, the activity of the catalase was found to increase significantly.

Catalase decomposes hydrogen peroxide and helps to protect from highly reactive hydroxyl radicals. Wang *et al*.[37] showed that, in Douchi extracts treated, cholesterol-fed rats, CAT activity in the liver, increased significantly compared to the negative control group. *Aphanamixis polystachya* bark extract supplementation resulted in dose-dependent and significant improvement in hepatic catalase level in rats.[38]

The treatment with the methanolic extract of *Sapindus emarginatus* pericarp caused an increase in the activities of CAT in the liver of the Wistar albino rats subjected to hepatotoxicity by anti-tubercular drugs rifampicin and isoniazid.[39]

The administration of green tea extract to the CCl_4 treated Wistar albino rats protected the liver from damage by increasing the activities of CAT and SOD.[40] An increase in SOD, CAT and GSH were observed in the liver and serum of the *Schistosoma mansoni* infected CD-1 Swiss albino mice after treatment with curcumin.[41]

Therefore, with the support of the above cited literatures our results also showed that the methanol extract of whole leaf and inner gel of *in vitro* and *in vivo* grown *Aloe vera* L., increased catalase enzyme activity which shows that the extracts can protect the normal cells from oxidative injuries.

Peroxidase (POD)

Peroxidase (POD) catalyses the reaction of conversion of hydrogen peroxide to oxygen and water. Several peroxidases are present in the biological system.[42] CAT and GPx are involved in the elimination of H_2O_2.

In the present study, a decrease in POD activity was observed in H_2O_2 treated groups. When methanol extract of either the whole leaf or only gel from *in vitro* and *in vivo* grown *Aloe vera* L. were co-administered this oxidative damage caused by H_2O_2 was reverted back to normal values which accounts for the antioxidant influencing activity of the plant.

The treatment with the antioxidant tocopherol caused an increase in the activities of peroxidase in the liver and kidney of hepatoxicity induced Wistar rats.[43] The antioxidant enzymes CAT and GPx have been reported to protect SOD against inactivation by H_2O_2.[44] The oral administration of methanolic extract of *Phyllanthus* significantly elevated the activity of hepatic glutathione peroxidase during CCl_4 stress in rats.[45]

Li *et al*.[46] reported that saponins from *Panax japonicus* protected against alcohol-induced hepatic injury in mice by up-regulating the expression of GPx, and SOD. The statistically significant losses in the activities of SOD, CAT and Px in acetaminophen-induced liver damage in mice was counteracted by all the tested plants (*Kigelia africana*, *Calotropis procera*, *Hibiscus sabdariffa* and *Alchornea cordifolia*) except *Calotropis procera* as reported by Olalye and Rocha.[47]

From the above discussed findings, it is obvious that the treatment with the extract significantly increased the activities of SOD, CAT and POD, which form the first line of defense against ROS.

Glutathione S-transferase (GST)

Glutathione S-transferases (GST's) are regulatory antioxidant enzymes involved in various cellular responses. They directly interact with the cellular enzymes and proteins which are involved in the protection against ROS and RNS. This enzyme is also involved in the regulation of cell proliferation, differentiation and apoptosis.[48]

Our data revealed that the exposure to H_2O_2 caused a steep decrease in GST activity compared to the control. This effect was effectively counteracted by the treatment with methanol extract of whole leaf and inner gel of *in vitro* and *in vivo* grown *Aloe vera* L., which showed an increase in GST activity. This improvement may have resulted from a change in the tissue redox system by scavenging free radicals.

Lee *et al*.[49] showed that pre-treatment of rats with tea seed oil (*Camellia oleifera* Abel.) could increase the activities of glutathione peroxidase, glutathione reductase and glutathione S-transferase in liver when compared with CCl_4-treated group.

The administration of curcumin and vitamin E increased the activities of SOD, CAT, GPx, GR and GST in the liver of CCl_4 intoxicated Wistar albino rats[50] Treatment with the flavonoid rutin increased the activities of SOD, CAT and GST in the liver of hepatoxicity induced Swiss albino mice[51] The administration of silymarin and commercially available *Withania somnifera* protected the Wistar rat liver from paracetamol induced toxicity as evidenced by increase in the activities of SOD, CAT and GST.[52]

All these above discussed findings supported our outcomes that the methanol extract of either the whole leaf or only gel from *in vitro* and *in vivo* grown *Aloe vera* L., effectively render protection to liver slices from oxidant mediated damage.

Glutathione reductase (GR)

Glutathione reductase (GR) is an antioxidant defence enzyme, which catalyses the reduction of glutathione, which is involved in bactericidal action of phagocytes.[53] Singh *et al*.[54] reported that the treatment with methanolic extract of *Daucus carota* seeds increased the activity of SOD, CAT, GR, GPx and GST in liver

homogenate of thioacetamide treated groups. The active compound paeonol, when administered to the hepatocellular carcinoma induced rat models, showed an increase in the activities of enzymic antioxidants SOD, CAT, GPx and GR in the liver.[55]

Our result also correlates with the above-mentioned findings. H_2O_2 treatment caused a decrease in the activity of GR whereas treatment with the methanol extract of either the whole leaf or only gel from *in vitro* and *in vivo* grown *Aloe vera* L., effectively relieved the oxidative stress cause by H_2O_2 and increased the GR activity.

Thus, our results of the activities of enzymatic antioxidants were supported by several studies conducted by other researchers as mentioned above. Overall, the results of the current study with goat liver slices as an *in vitro* model subjected to oxidative stress and treated with the methanol extract of the *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and inner gel showed that the extracts are able to protect the liver cells from oxidative damage and also do not induce any harmful effects in the liver slices.

CONCLUSION

Leaf extracts of *Aloe vera* L. (whole leaf and inner gel of *in vivo* grown and *in vitro* regenerated) were assessed for their enzymatic antioxidant potentials and it was concluded that *Aloe vera* L. showing significant enzymatic antioxidant activities. In present investigation enzymatic antioxidant potential of the *Aloe vera* L. were studied using *in vitro* models (alternatives to the live animals) which mimics the *in vivo* conditions to avoid the use of live animals and less harm them. For this study, precision cut liver slices were used as an *in vitro* model. The liver slices were subjected to oxidative stress and the effect of methanol extract of *Aloe vera* L. whole leaves and inner gel in counteracting this stress was assessed by analysing the enzymatic antioxidant status and it was found that methanol extract of *in vitro* and *in vivo* grown *Aloe vera* L. whole leaf and only gel samples were showing the significant antioxidant activities. Therefore, the results of the current investigation confirmed the enzymatic antioxidant capacity of the *Aloe vera* L. leaves. All the samples of leaf extracts were able to relieve the liver slices from the oxidative stress induced by H_2O_2. Thus, all the findings also revealing that these enzymatic antioxidant principles of *Aloe vera* L. leaves can fight against the free radical or oxidant mediated disorders.

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