Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 5, 2015

Original Article

IN VITRO ANTIOXIDANT ACTIVITY OF *PROSOPIS CINERARIA* LEAVES AGAINST H_2O_2 INDUCED OXIDATIVE STRESS IN GOAT LIVER SLICES

DHARANI BANGARUSWAMY[a], SIVAPRABHA JAMBUNATHAN[b], PALGHAT RAGHUNATHAN PADMA[a], SUMATHI SUNDARAVADIVELU[a]*

[a]Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore 641043, Tamil Nadu, India, [b]Department of Biotechnology, K. S. Rangasamy college of Arts and Science, Tiruchengode, Tamil Nadu, India
Email: sumathi_vnktsh@yahoo.co.in

Received: 29 Jan 2015 Revised and Accepted: 24 Feb 2015

ABSTRACT

Objective: To evaluate the antioxidant status of goat liver slices treated with standard oxidant H_2O_2in the presence and the absence of methanolic leaf extract of *Prosopis cineraria*.

Methods: Precision-cut goat liver slices were challenged with oxidant H_2O_2 with or without the methanolic leaf extract of *Prosopis cineraria*. The activity of enzymic antioxidants and the levels of non-enzymic antioxidants were analysed in the homogenate of liver slices.

Results: The levels of antioxidants were drastically decreased in oxidant treated group compared to untreated control, whereas there was a significant increase in the antioxidants levels in the methanolic leaf extract treated group.

Conclusion: The organ slices represent the multicellular, structural and functional features of *in vivo* tissues and therefore represent as a good *in vitro* model. The present study revealed that leaf extract improved the antioxidant status of the goat liver slices and also protected against oxidative damage.

Keywords: *in vitro* models, *Prosopis cineraria*, Liver slices, Antioxidant activity, Hydrogen peroxide, Oxidative stress, Vanni, Enzymic antioxidants, Non-enzymic antioxidants.

INTRODUCTION

Free radicals are species that contains one or more unpaired electrons, which make them very unstable and, therefore, to attain stability, they extract electrons from other molecules, thereby causing damage to them [1]. During oxidative stress conditions, there is an imbalance between antioxidants and reactive oxygen species; hence, exogenous supply of antioxidants is important to prevent the body from free radical-induced injury [2]. Oxidative stress is considered as a major etiological and/or pathogenic agent of most degenerative diseases such as cancer, Alzheimer's disease, diabetes and aging [3]. Antioxidants are substances that inhibit and scavenge radicals and thus protect humans against infectious and degenerative diseases [4]. Natural antioxidants scavenge the excessively generated free radicals in the human body and combat the devastating consequences of free radicals. Hence, they are considered to be very useful therapeutic agents in a large variety of human diseases [5]. Development of *in vitro* model systems is very important in order to evaluate the chemical and drug toxicity, reduce the cost of animal testing and also to reduce the sufferings of animals [6]. Among the *in vitro* models, precision-cut liver slices are very useful for studying drug metabolism and toxicity [7]. Medicinal plants are a rich source of bioactive phytochemicals or bionutrients. The recent trend has moved towards the use and ingestion of natural antioxidants present in different parts of plants due to their phytochemical constituents [8].

The candidate plant chosen for the present study is *Prosopis cineraria,* commonly called as Vanni (Tamil) and Jhand tree (Hindi). The tree belongs to *Fabaceae* family and is used as a remedy for cough, cold, asthma and rheumatism. The plant also possesses anti-inflammatory property. Previous study proved that the methanolic leaf extract of *Prosopis cineraria* is a potential free radical scavenger. Therefore, the present study focused on evaluating the antioxidant status of oxidatively stressed goat liver slices in the presence and the absence of methanolic leaf extract of *Prosopis cineraria*.

MATERIALS AND METHODS

Preparation of the extract

1g of fresh leaf sample and 10 ml of methanol were homogenized in a mortar and pestle. The extract was then centrifuged at 2000 rpm for 5 minutes and the methanol was dried at 60°C protected from light. The resulted residue was weighed and dissolved in dimethyl sulfoxide (DMSO) at a concentration of 20 mg/20 µl. Free radical scavenging activity was performed with different concentrations and the dose of 0.15 mg/5 µl of DMSO were optimized. Hence, the same dose was used for assessing enzymic andnon-enzymic antioxidants in *in vivo* stimulated *in vitro* model.

Preparation of goat liver homogenate

The liver was purchased fresh from the local slaughter house. The tissue was quickly plunged into cold PBS and maintained at 4ºC. The tissue was sliced into 1 mm thin slices by using the sterile scalpel. 250 mg of tissue was taken in 1.0 ml of cold PBS. The H_2O_2 (5 µl) and methanolic extract (5 µl) were added and incubated at 37ºC for one hour with mild shaking. After the incubation period, the tissues were homogenized in the same aliquot of the PBS using a Teflon homogenizer and centrifuged to remove the debris. The supernatant was then used for the estimation of various parameters to assess the antioxidant potential. The concentration of H_2O_2 used was 200µM. The experimental groups set up for the study were as follows.

1) Untreated (negative) control.

2) H_2O_2 treated (positive) control.

3) Methanolic extract of *Prosopis cineraria* leaves

4) Methanolic extract of *Prosopis cineraria* leaves+H_2O_2

Parameters analysed

The antioxidant effect of *Prosopis cineraria* leaves was assessed by analyzing activities of enzymic antioxidants and the levels of non-enzymic antioxidants.

Determination of the activity of enzymic antioxidants

i) Assay of superoxide dismutase (SOD)

Superoxide dismutase activity was assayed spectrophotometrically by the method of Misra and Fridovich (1972) [9]. The reaction

mixture contained 50 mm potassium phosphate buffer, 45µM methionine, 5.3µM riboflavin, 84µM NBT and 20 mm potassium cyanide and 100 µl of enzyme source at a final volume of 3 ml. The tubes were maintained at 25ºC equipped with 15W fluorescent lamps in an aluminium foil-lined box. After incubating for 10 minutes, reduced NBT was measured spectrophotometrically at 600 nm. The maximum reduction was observed in the absence of the enzyme source. One unit of enzyme activity was defined as the amount of enzyme giving a 50% inhibition of the reduction of NBT.

ii) Assay of catalase (CAT)

Catalase activity was determined by adopting the method of Luck (1974) [10]. Into quartz cuvette, 3 ml of H_2O_2 in phosphate buffer (0.067M, pH 7.0) was taken and the baseline was adjusted at 240 nm followed by 20µl of homogenate and mixed thoroughly. The time interval for decrease in absorbance by 0.05 units was recorded at 240 nm. The enzyme source and phosphate buffer without H_2O_2 served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units.

iii) Assay of peroxidase (POD)

The activity of peroxidase in the samples was assessed by the method of Reddy *et al.* (1995) [11]. Pyrogallol solution (3.0 ml) and enzyme extract (0.1 ml) were pipetted out into a cuvette. The spectrophotometer was adjusted to read zero at 430 nm followed by the addition of 0.5 ml of 1% H_2O_2 and mixed. The change in absorbance was recorded every 30 seconds up to 3 minutes. One unit of peroxidase activity is defined as the change in absorbance per minute at 430 nm.

iv) Assay of glutathione reductase (GR)

The activity of glutathione reductase was determined by the method of David and Richard (1983) [12]. The assay system contained 1 ml of potassium phosphate buffer (0.12M, pH7.2), 0.1 ml EDTA (15 mm), 0.1 ml sodium azide (10 mm), 0.1 ml of oxidized glutathione (6.3 mm) and 0.1 ml of liver homogenate. The final volume was made up to 2 ml using water and incubated for 3 minutes. Following incubation, 0.1 ml of 6.3 mm NADPH was added. The absorbance at 340 nm was recorded at an interval of 15 seconds for 2-3 minutes. One unit of enzyme activity was expressed as µmoles of NADPH oxidized/minute/g liver tissue.

v) Assay of glutathione S-transferase (GST)

The assay of glutathione S-transferase activity was performed by the method of Habig *et al.* (1974) [13]. The enzyme activity was determined by the change in absorbance at 340 nm in a spectrophotometer. The reaction was started by the addition of 0.1 ml of liver homogenate to the mixture containing 0.1 ml of GSH, 0.1 ml of CDNB and phosphate buffer in a total volume of 2.9 ml and the readings were recorded for a minimum of three minutes. Distilled water served as a blank and the complete assay mixture without the enzyme served as the control to monitor non-specific binding of the substrates. One unit of GST activity is defined as the nmoles of CDNB conjugated per minute.

Determination of the levels of non-enzymic antioxidants

i) Estimation of ascorbic acid

Ascorbic acid present in the sample was estimated by the method of Roe and Keuther (1943) [14].0.2 to 1.0 ml of the working ascorbate solution and 0.1 ml of the liver homogenate were taken. The reaction mixture was made up to 2.0 ml with 4% TCA. To this, 0.5 ml DNPH reagent was added followed by two drops of 10% thiourea solution. The tubes were incubated at 37ºC for 3 hours. The osazone crystals formed were dissolved by the addition of 2.5 ml 85% H_2SO_4. DNPH reagent and thiourea were added to blank after the addition of H_2SO_4. After cooling the tubes, the absorbance was read at 540 nm.

A standard graph was constructed using an electronic calculator set to the linear regression mode. The concentration of ascorbate in the samples were calculated and expressed in terms of mg/g of sample.

ii) Estimation of tocopherol

The levels of tocopherol in the samples were estimated spectrophotometrically by the method reported by Rosenberg (1992) [15]. The liver homogenate, standard and water of 1.5 ml were pipetted out into three centrifuge tubes namely test, standard and blank respectively. To all the tubes, 1.5 ml ethanol and xylene were added, stoppered, mixed well and centrifuged. After centrifugation, the xylene layer was transferred into another tube, taking care not to include any ethanol or protein. To 1.0 ml of xylene layer, 1.0 ml of 2, 2′-dipyridyl reagent was added and mixed. This reaction mixture was taken in the spectrophotometric cuvettes and the extinctions of the test and the standard were read against the blank at 460 nm. Then, 0.33 ml of ferric chloride solution was added, mixed well and after exactly 15 minutes, the test and the standard were read against the blank at 520 nm. The levels of tocopherol were calculated using the formula

$$\text{Tocopherols}(\mu g) = \frac{\text{Sample A520} - \text{A460}}{\text{Standard A520}} \text{ X } 0.29 \text{ X } 0.15$$

iii) Estimation of vitamin A

The estimation of vitamin A in goat liver homogenate in the presence and the absence of leaf extracts were done by the method proposed by Bayfield and Cole (1980) [16]. One ml of liver homogenate was mixed with 1.0 ml of saponification mixture (2N KOH in 90% alcohol) and refluxed for 20 minutes at 60ºC in the dark. All the steps subsequent to saponification were carried out in the dark. Vitamin A was extracted twice with 10 ml of petroleum ether (40-60ºC). The extracts were pooled, washed thoroughly with water and the layers were separated using a separating funnel. When the petroleum ether fraction was clear, a pinch of sodium sulphate (anhydrous) was added to remove the excess moisture. The volume of the extract was noted and the fraction (1.0 ml) was evaporated to dryness at 60ºC. The residue was dissolved in 1.0 ml of chloroform. Aliquots of the standard (vitamin A palmitate) were pipetted out into a series of clean, dry test tubes in the concentration ranging from 0 to 7.5µg. The volume in all the tubes was made up to 1.0 ml with chloroform. TCA reagent (2.0 ml) was added rapidly, mixed well and the absorbance of blue colour was read immediately at 620 nm in a spectrophotometer. The vitamin A level was expressed as µg/g tissue.

iv) Estimation of reduced glutathione

The levels of reduced glutathione were estimated by the method proposed by Moron *et al.* (1979) [17]. To 0.5 ml of the liver homogenate 2.5 ml of 5% TCA was added. The precipitated protein was centrifuged at 1000rpm for 10 minutes. The supernatant (0.1 ml) was used for the estimation of GSH.

0.1 ml of the sample was made up to 1 ml with 0.2M sodium phosphate buffer (pH 8). 2.0 ml of freshly prepared DTNB solution (0.6M in phosphate buffer) was added and the intensity of the yellow colour formed was read at 412 nm in a spectrophotometer after 10 minutes. A standard curve of GSH was prepared using concentrations ranging from 2-10 micromoles of GSH. The values are expressed as n moles of GSH/g tissue.

v) Determination of total thiols and protein thiols

The protein thiols and total thiols were estimated as per the procedure outlined by Sedlack and Lindsey (1968) [18].1.0g of the tissue was homogenized with 5 ml of 0.2M Tris-EDTA (pH 8.0). The assay mixture contained 0.2 ml of the homogenate, 1.5 ml of buffer (0.2M tris, pH 8.0, containing 0.2M EDTA) and 1.0 ml of DTNB. This mixture was then made upto 10.0 ml with absolute methanol. Two tubes containing a reagent blank without sample and another without DTNB were stoppered and allowed to stand for 15 minutes with occasional shaking at room temperature. The tubes were then centrifuged at 3000g for 15 minutes and absorbance was measured at 420 nm in a spectrophotometer. The level of total thiols minus glutathione levels gives the amount of protein thiols in the tissue. Protein thiols are expressed as mg/g tissue.

Statistical analysis

Values were expressed as mean±SD. Statistical difference in mean was analysed using one way ANOVA and followed by least square mean deviation comparison tests (LSD). $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Enzymic antioxidants

The results of the present study showed that the activities of enzymic antioxidants significantly decreased in the H_2O_2 exposed group when compared to the untreated control group. Treatment with the methanolic extract of *Prosopis cineraria* leaf caused a significant increase in the antioxidant status when compared to the untreated control group. In the oxidant and plant extract treated group, the antioxidant levels were higher than the oxidant alone treated group, which indicated the protective role of the leaf extract in oxidatively stressed goat liver slices. The activities of enzymic antioxidants are represented in table 1.

Table 1: It shows the effect of *Prosopis cineraria* leaf extract on enzymic antioxidant activities in goat liver slices exposed to H_2O_2 *in vitro*

Enzymic Antioxidants (U/g tissue)	Treatment groups			
	Untreated control	H_2O_2 treated	Leaf extract treated	H_2O_2+leaf extract treated
SOD[1]	14.48±0.44	11.06±0.67[a]	15.89±0.66[a]	13.43±0.08[a, b, c]
CAT[2]	360.00±28.28	138.9±4.08[a]	445.23±35.04[a]	256.33±23.35[a, b, c]
POD[3]	29.62±1.50	27.02±0.20[a]	31.98±0.80[a]	30.56±0.45[b, c]
GST[4]	0.153±0.01	0.080±0.01[a]	0.199±0.05[a]	0.12±0.01[a, b, c]
GR[5]	1.53±0.06	1.2±0.00[a]	2±0.10[a]	1.37±0.06[a, b, c]

Values are mean±SD of triplicates

Units

1-1 Unit is the amount of enzyme that gave 50% inhibition of the extent of NBT reduction in 1 minute

2-1Unit is the amount of enzyme required to decrease the absorbance by 0.05 units at 240 nm

3-1 Unit is the change in absorbance at 430 nm per minute.

4-1 Unit is the milli moles of NADPH oxidized/minute

5-1 Unit is the nano moles of CDNB conjugated/minute.

Group comparison

a-statistically significant ($p<0.05$) compared to untreated control

b-statistically significant ($p<0.05$) compared to H_2O_2 control

c-statistically significant ($p<0.05$) compared to the respective plant control

Similar results have been reported by Nandy *et al.* (2012) [19] whose observations showed that the methanolic extract of *Leucas plukenetti* Smith. whole plant enhanced the CAT, SOD and GSH activities in the goat liver slices. The decreased levels of SOD, CAT, GSH and GPx in streptozotocin induced rats were significantly increased after the administration of methanolic extract of *Coleus vettiveroides*Jacob whole plant [20]. Goat liver slices treated with different extracts of M*oringa oleifera* showed a significant increase in the CAT activity on exposure to CCl_4 [21].

In another study, Sridharan *et al.* (2014) [22] reported that EAC inoculation in mice resulted in the marked elevation of LPO and reduced levels of non-enzymic (GSH) and enzymic antioxidants (GPX, SOD and catalase) in the hepatic tissue. The water extract of aerial parts of *Tecomastans* (L). Juss. ex. Kunth., when administered, effectively restored the antioxidant system.

Saradhamani (2011) [23] reported that the methanolic extract of *Majorana hortensis* leaves improved the activities of SOD, CAT, POD and GST activities of liver slices exposed to hydrogen peroxide. Sivaprabha *et al.* (2012) [24] reported that the exposure of goat liver slices to H_2O_2 decreased the activities of enzymic antioxidants namely SOD, CAT, perioxidase, GST and GR. The presence of leaf and rhizome extracts of *Curcuma amada* increased the activities of enzymic antioxidants compared to oxidant treated group. Kalaiselvi *et al.* (2011) [7] stated that the methanolic extract of *Jasminum sambac* (Linn) flowers increased the activities of SOD, CAT, GPx, GR and vitamins C, E, A and GSH levels in the liver slices exposed to H_2O_2. The administration of CCl_4 in rats caused a decrease in the CAT, POD and SOD activities and this was effectively restored on treatment with the methanol, hexane and ethyl acetate extract of *Carissa opaca* fruits [25]. Devi and Vasuki (2009) [26] reported that *Rhinacanthus nasutus* leaf extract improved the antioxidant activities of H_2O_2 exposed goat liver slices. The study by Latha and Latha (2013) [27] reported that *Leucas aspera* methanolic extract improved the hepatic GSH, GST, GPx, SOD and catalase activities in CCl_4 induced rats. *Lophira alalata* stem bark methanol extract increased the liver SOD, CAT, GPx and GR activities in a dose dependent manner [28].

Non-enzymic antioxidants

The levels of non-enzymic antioxidants namely vitamins C, E, A, GSH, total and protein thiols are presented in table 2. From table 2, it is clear that exposure to hydrogen peroxide caused a significant reduction in the levels of non-enzymic antioxidants. A significant increase in the level of non-enzymic antioxidants was observed on administration of the methanolic extract of the leaves. When the tissue slices were exposed to both the oxidant and the methanolic leaf extract of *Prosopis cineraria*, there was a significant improvement in the levels of the non-enzymic antioxidants.

The results correlate with that of several studies reported. Goat liver slices treated with H_2O_2 showed depletion of vitamin C and the effect was counteracted by treatment of methanolic leaf extract of *Nyctanthes arbor-tristis* [29]. Ramesh and Satakopan (2010) [30] reported that the methanolic leaf extract of *Ocimum sanctum* increased the liver ascorbate levels in cadmium treated rats. Pradeep*et al.* (2010) [31] reported that the decreased level of vitamin C, vitamin E and GSH on ethanol and diethylnitrosamine exposure in liver of the albino Wistar rats, was improved on co-administration of ethanolic leaf extract of *Cassia fistula*.

Yamuna and Padma (2013) [32] reported that there was a signi ficant increase in the levels of vitamins C, E, A and GSH in the goat liver slices exposed to H_2O_2 after treatment with the methanolic extract of *Caesalpinia pulcherrima* flowers. Palan is wamy and Padma (2011) [33] have reported that there was a significant reduction in vitamins C, E and A levels in H_2O_2 challenged liver slices. The methanolic and the aqueous extract of *Majorana hortensis* leaves were able to revert this reduction.

Haniya and Padma (2013) [34] reported that hydrogen peroxide caused a decline in the vitamin a level in goat liver slices and the methanolic leaf extract of *Artemisia vulgaris* effectively reverted the antioxidant level. Kavitha (2013) [35] reported that the chloroform extract of *Costus pictus* leaves increased the levels of vitamin C, A, E and GSH of goat liver slices exposed to hydrogen peroxide. Similarly H_2O_2 treatment showed vitamin A depletion in goat liver slices. Co-treatment of *Zea mays* leaf extracts reverted this depletion in leaf extract treated group and also in H_2O_2 treated group [36]. Padma and Kalaivani (2013) [37] reported that the exposure of goat liver slices to CCl_4 caused a decrease in the levels of vitamin C, E, A and GSH and co-exposure to the *Solanum nigrum* leaves increased the levels of these non-enzymic antioxidants.

Morrone *et al.* (2013) [38] proved that the aqueous extract of *Passiflora manicata* leaves reverted the total protein thiols oxidation

in hydrogen peroxide treated rat brain and rat liver slices. The decreased level of hepatic total thiols, CAT and GST in CCl_4 induced albino rats were increased on pre-treatment with the aqueous bark extract of *Terminalia paniculata* [39]. The level of total thiols was significantly decreased upon paracetamol treatment. The co-administration of methanolic extract of *Amaranthus viridis* reverted the effect [40]. Treatment with *B. monnieri* leaf extracts minimized the effects of CCl_4 in the liver and kidney of rats [41]. Theco-administration with artemisinin and dihydroartemisinin caused a significant increase in the total and protein thiol levels compared to H_2O_2 treated goat liver slices [42].

The above findings strength en our findings that the methanolic leaf extract of *P. cineraria* is capable of improving the antioxidant levels.

Table 2: It shows the effect of *Prosopis cineraria* leaf extract on non-enzymic antioxidant levels in goat liver slices exposed to H_2O_2 *in vitro*

Non-enzymic Antioxidants (U/g tissue)	Treatment Groups			
	Untreated control	H_2O_2 treated	Leaf extract treated	H_2O_2+leaf extract treated
Vitamin C (mg/g tissue)	0.62±0.01	0.57±0.05[a]	0.96±0.01[a]	0.77±0.09[a, b, c]
Vitamin E (µg/g tissue)	11.17±0.44	6.88±0.78[a]	13.32±0.34[a]	9.04±1.07[a, b, c]
Vitamin A (µg/g tissue)	72.01±0.33	54.53±0.44[a]	89.64±1.75[a]	72.55±1.31[a, b, c]
GSH (mmoles/g tissue)	5.92±0.05	3.95±0.05[a]	6.41±0.03[a]	5.46±0.03[a, b, c]
Total thiols (mg/g tissue)	33.30±0.27	22.20±0.27[a]	36.08±0.17[a]	30.74±0.16[a, b, c]
Protein thiols (mg/g tissue)	31.48±0.26	20.99±0.26[a]	34.11±0.16[a]	29.06±0.15[a, b, c]

Values are mean±SD of triplicates

CONCLUSION

The results of the study with goat liver slices, confirm that the methanolic extract of *P. cineraria* leaves can improve the antioxidant status of the tissue by increasing the activities/levels of both enzymic and non-enzymic antioxidants.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Soni A, Sosa S. Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. J Pharmacogn Phytochem 2012;2:22-9.
2. Thatoi HN, Patra JK, Das SK. Free radical scavenging and antioxidant potential of mangrove plants: a review. Acta Physiol Plant 2014;36:56-579.
3. Davila JC, Rodriguez JR, Melchert BR, Daniel Acosta. Predictive value of *in vitro* model systems in toxicology. Annu Rev Pharmacol Toxicol 1998;38:63-96.
4. Imran I, Zia-Ul-Haq M, Calani L, Mazzeo T, Pellegrini N. Phenolic profile and antioxidant potential of selected plants of Pakistan. J Appl Bot Food Qual 2014;87:30-5.
5. Alok S, Jain SK, Verma A, Kumar M, Mahor A, Sabharwal M. Herbal antioxidant in clinical practice: A review. Asian Pac J Trop Biomed 2014;4:78-84.
6. Lerche-Langrand C, Toutain HJ. Precision-cut liver slices: characteristics and use for *in vitro* pharmaco-toxicology. Toxicol 2000;153:221-53.
7. Kalaiselvi M, Narmadha R, Ragavendran P, Raj A, Sophia D, Kumar GR, *et al. In vivo* simulated *in vitro*model of *Jasminum sambac* (Linn.) using mammalian liver slice technique. Asian Pac J Trop Biomed 2011;1:S216-S219.
8. Mbaebie BO, Edeoga HO, Afolayan AJ. Phytochemical analysis and antioxidants activities of aqueous stem bark extract of *Schotialatifolia*. Asian J Exp Biol Sci 2012;2:118-24.
9. Misra HP, Fridovich I. The role of superoxide anion in the antioxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972;247:3170-5.
10. Luck H. In: Methods in enzymatic analysis. II Edn. Bergmeyer, Academic Press: New York; 1974. p. 805.
11. Reddy KP, Subhani SM, Khan PA, Kumar KB. Effect of light and benzyladenine on dark treated growing rice (*Oryzasativa*) leaves-changes in peroxidase activity. Plant Cell Physiol 1995;26:987-94.
12. David M, Richard JS. In: Methods of enzymatic analysis. Bergmeyer J, Marianne Grab. eds. Verlag Chemie Weinheim Deer Field, Beach Floride; 1983. p. 358.
13. Habig WH, Pabst MJ, Jokoby WB. Glutathione transferase: A first enzymatic step in mercapturic acid III formation. J Biol Chem 1974;249:7130-9.
14. Roe JH, Kuether CA. The determination of ascorbic acid in whole blood and urine through 2, 4-dinitrophenylhydrazine derivative dehydro ascorbic acid. J Biol Chem 1943;147:399-407.
15. Rosenberg HR. Chemistry and physiology of the vitamins. Interscience Publisher: New York; 1992. p. 452-3.
16. Bayfield RF, Cole ER. Colorimetric estimation of vitamin a with trichloroacetic acid. Methods Enzymol 1980;67:189-95.
17. Moron MS, Depierre JN, Mannervik VC. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochem Biophys Acta 1979;582:67-8.
18. Sedlack J, Lindslay RH. Estimation of total protein and non-protein sulphydryl groups in tissues with Ellman's reagent. Anal Biochem 1968;25:192-8.
19. Nandy S, Paul HS, Barman NR, Chakraborty B. *In vitro* evaluation of antioxidant activity of *Leucasplukenetii* (Roth) Spreng. Asian J Plant Sci Res 2012;2:254-62.
20. Gopalakrishnan G, Dhanapal CK. Evaluation of *in-vivo* antioxidant activity of methanolic extract of *Coleus vettiveroides* Jacob in streptozotocin-induced oxidative stress in rats. Int J Pharm Pharm Sci 2014;6:590-2.
21. Sreelatha S, Padma PR. Protective mechanisms of *Moringaoleifera* against CCl_4-induced oxidative stress in precision-cut liver slices. Forsch Complement Med 2010;17:189-94.
22. Sridharan G, Sarvanan R, Brindha P. Evaluation of anticancer potentials of *Tecomastans* (L). Juss. Ex. Kunth against EAC cell lines. Int J Pharm Pharm Sci 2014;6:88-92.
23. Saradhamani KR. Evaluation of antioxidant activity of *Majoranahortensis* leaf extract on goat liver slices subjected to oxidative stress, A thesis submitted to the Avinashilingam Deemed University for Women for the award of Master of Science in Biotechnology; 2011.
24. Sivaprabha J, Dharani B, Padma PR, Sumathi S. *In vitro* prevention of oxidative damage by *Curcuma amada* in goat liver slices exposed to oxidative stress. J Pharm Res 2012;5:1108-11.
25. Sahreen S, Khan MR, Khan RA. Effects of *Carissa opaca* fruits extracts on oxidative pulmonary damages and fibrosis in rats. BMC Complem Altern Med 2014;14.
26. Devi RN, Vasuki D. Effect of *Rhinacanthus nasutus extracts* on the non-enzymic antioxidant status of oxidant induced goat liver slices. Asian J Bio Sci 2009;2:149-52.
27. Latha B, Latha MS. Antioxidant and curative effect of *Leucasaspera*methanolic extract against carbontetra chloride induced acute liver injury in rats. Int J Pharm Pharm Sci 2013;5:374-8.
28. Ajiboye TO, Yakubu MT, Oladiji AT. Electrophilic, free radical and reactive oxygen species scavenging and detoxification potentials of *Lophiraalata*stem bark extract. Free Rad Antiox 2011;1:40-7.

29. Sumathi S, Sowmini CM, Dharani B, Sivaprabha J, Padma PR. Antioxidant status of goat liver slices treated with methanolic leaf extract of *Nyctanthesarbor-tristis* exposed to oxidative stress. Int J Pharm Biosci 2011;2:376-82.
30. Ramesh B, Satakopan VN. Antioxidant activities of hydroalcoholic extract of *Ocimum sanctum* against cadmium induced toxicity in rats. Indian J Clin Biochem 2010;25:307-10.
31. Pradeep K, Mohan VCR, Gobianand K, Karthikeyan S. Protective effect of *Cassia fistula* Linn. ondiethylnitrosamine induced hepatocellular damage and oxidative stress in ethanol pretreated rats. Biol Res 2010;43:113-25.
32. Yamuna ST, Padma PR. Antioxidant potential of the flowers of *Caesalpiniapulcherrima*, Swartz in an *in vitro* system subjected to oxidative stress. J Pharm Res 2013;7:661-5.
33. Palaniswamy R, Padma PR. Antioxidant activity of *Majoranahortensis* leaves subjected to oxidative stress in an *in vitro* system. Int Res J Pharm 2011;2:153-7.
34. Haniya AMK, Padma PR. Antioxidant effect of *Artemisia vulgaris* leaf extracts on oxidatively stressed precision-cut goat liver slices. Int Res J Pharm 2013;4:55-60.
35. Kavitha R. *In vitro* antioxidant studies of *Costuspictus* leaf. Int J Res Pharm Biomed Sci 2013;4:159-64.
36. Balasubramanian K, Padma PR. Protection of precision cut-goat liver slices by *Zea mays* leaf extracts from hydrogen peroxide induced oxidative stress *in vitro*. Int J Pharm Bio Sci 2012;3:201-10.
37. Padma PR, Kalaivani K. *Solanum Nigrum*leaves improve the oxidative status of liver tissue *in vitro*. India J Nutr Dietiet 2013;50:440-9.
38. Morrone MS, De Assis AM, Da Rocha RF, Gasparotto J, Gazola AC, Costa GM, *et al. Passifloramanicata* (Juss.) aqueous leaf extract protects against reactive oxygen species and protein glycation*in vitro* and *ex vivo* models. Food Chem Toxicol 2013;60:45-51.
39. Talwar S, Jagani HV, Nayak PG, Kumar N, Kishore A, Bansal P, *et al.* Toxicological evaluation of *Terminaliapaniculata* bark extract and its protective effect against CCl_4-induced liver injury in rodents. BMC Complem Altern Med 2013;13:127.
40. Kumar BSA, Lakshman K, Swamy VBN, Kumar PAA, Shekar DS, Manoj B, *et al.* Hepatoprotective and antioxidant activities of *Amaranthusviridis* Linn. Macedonian. J Med Sci 2011;4:125-30.
41. Radha P. Antioxidant responses evoked *in vitro* by *Bacopamonnieri* leaf extracts. A thesis submitted to Avinashilingam University for Women, Coimbatore in partial fulfilment of the requirement for the degree of Doctor of Philosophy in Biochemistry; 2010.
42. Ramya N. Comparison of the antioxidant efficacy of the active components of Artemisia vulgaris using goat liver slices as an *in vitro* model. A thesis submitted to Avinashilingam Deemed University for Women, Coimbatore in partial fulfilment of the requirements for the degree of Master of Science in Biochemistry; 2012.