Asian Pub. Corp.

www.asianpubs.org

ARTICLE

Antioxidant Activity of 2-Bromo-3-hydroxy-2-nitropropylcinnamate against Alcohol Induced Oxidative Damage in Rats

Rajesh Kumar Malik[1], Dharmendra Kumar Singh[2], Anuradha[3] and Surendra Kumar[1,✉]

Asian Journal of Organic & Medicinal Chemistry

Volume: 5 Year: 2020
Issue: 2 Month: April–June
pp: 156–160
DOI: https://doi.org/10.14233/ajomc.2020.AJOMC-P266

Received: 24 April 2020
Accepted: 20 June 2020
Published: 2 July 2020

ABSTRACT

The present study demonstrated the antioxidant activity of 2-bromo-3-hydroxy-2-nitropropylcinnamate *versus* alcohol induced oxidative damage in albino wistar rats. In this study, 30 % alcohol exposed rats were found to be more prone to peroxidative risk as they are calculated by species of thiobarbituric acid. It was observed that after the rats induction with 30% alcohol, concentration of lipid peroxidation has been obtained expressively ($p \leq 0.001$) high in a liver and serum, beside with concomitant substantial ($p \leq 0.001$) reduced in enzymatic and non-enzymatic antioxidants levels like catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), vitamin E (tocopherol), reduced glutathione (GSH), glutathione-*s*-transferase (GST), vitamin C (ascorbic acid), β-carotene as well as ceruloplasmin in serum along with liver, only 30% alcohol was treated. If rats obtained 2-bromo-3-hydroxy-2-nitropropylcinnamate at the dose level of 20 mg/kg bw/day, PO, for 30 days, the peroxidative damage has been marginal in serum along with liver, alongside efficiently encouraging the potential of antioxidant in the rats treated by alcohol. This study revealed that in liver the raised peroxidative risk is probably allied with alcohol induction pathology that can be decreased by increasing the antioxidant potential by free radical scavenging activity, therefore promising as artificial antioxidants for 2-bromo-3-hydroxy-2-nitropropylcinnamate.

KEYWORDS

Lipid peroxidation, 2-Bromo-3-hydroxy-2-nitropropylcinnamate, Antioxidant activity.

INTRODUCTION

Antioxidants are the compounds which inhibits the oxidation. Any chemical reaction producing the free radicals, thereby leads to chain reaction which might harm the organism cells and is termed as oxidation. Thiols or ascorbic acids (vitamin C) terminates these chain reactions and acts as antioxidants. For balancing the oxidative stress, animals and plants maintains complex system of the overlapping antioxidants, like glutathione and enzyme such as superoxide mutase and catalase, produced internally or the dietry antioxidant vitamin E and vitamin C [1].

The antioxidants are commonly having two different groups of substrates in use; the industrial chemicals products for oxidation prevention, as well as the natural compounds

Author affiliations:

[1]Department of Chemistry, Maharshi Dayanand University, Rohtak-124001, India
[2]Department of Zoology, Dyal Singh College (University of Delhi), Lodhi Road, New Delhi-110003, India
[3]Department of Chemistry, Daulat Ram College (University of Delhi), Delhi-110007, India

✉To whom correspondence to be addressed:

E-mail: surendra.kumar85@gmail.com

Available online at: http://ajomc.asianpubs.org

present in the tissues as well as food. The industrial oxidants have varied utilization: performing as the preservatives in the cosmetics along with foods as well as in fuels as a oxidation-inhibitors [2]. The antioxidant dietry supplement has not demonstrated any improvement in human health or to be active in preventing the disease [2].

Antioxidants are classified as hydrophilic (soluble in water) and hydrophobic (insoluble in water). The hydrophilic antioxidant reacts with the oxidants present in blood plasma along with cell cytosol, whereas the cell membrane is prevented by hydrophobic antioxidants [3]. All these compounds can be produced in a body as well as could be found from food and other food products [4]. There are present different oxidants in the body fluids and tissues, example glutathione or ubiquinone which is mostly present in the cell, whereas few other like uric acid is uniformly distributed. In some organisms antioxidants are found as well as these compounds could be significant in the pathogens along with virulence factors [5]. Interaction among the antioxidants is such a complicated issue, having most of an antioxidant compounds as well as antioxidant enzyme system with interdependent along with synergistic effect on each other [6,7]. The mode of action of the antioxidant depends on the functioning of the all members of an antioxidant system [4]. The amount of protection delivered through any antioxidant can also be governed by its reactivity to the ROS being deliberated, the antioxidant as well as concentration status having its interacting [4].

Many of the compounds exhibits antioxidant defence by the chelating transition metals as well as inhibit them from catalyzing the production of free radical in a cell. There is extensive evidence to implicate free radicals in the recent research and development of many of the degeneration diseases [8]. The free radicals need to be implicated in causation of ailments like nephrotoxicity, liver cirrhosis, diabetes, *etc*. [9]. The free radicals intermediated lipid peroxidation calculated to become a main mechanism of cellular damage as well as cellular membrane devastation [10]. The most extensively examined instance will be the lipid peroxidation activated by hepatotoxin alcohol model. Generally, there seems in order to be rising evidence about alcoholic toxicity might be connected with free radical related injury as well as raised oxidative stress [11]. Production of oxygen metabolites including O_2^- (superoxide), H_2O_2 (hydrogen peroxide) as well as OH^- (hydroxyl radical) is thought to be essential in the pathogenesis of alcohol liver injury [12]. To deal with the oxidants, cells have many antioxidant enzymes like GPx, GSH, CAT, and SOD.

Recently, antioxidative activities of *N*-oxides of tertiary amines have been carried out [13]. Antioxidative kaempferol impacts as well as their equimolar mixture having phenyltin compounds has been also studied [14,15]. Souza and Giovani [16] demonstrated that complicated flavonoids has high impact as compared to free flavonoids. Further, it has been argued that the high antioxidant complexes′ activity is because of only adding superoxide dismutating centers [17]. Boadi *et al*. [18] have shown that the complexed flavonoid provide more protection as compared to single treatment as well as can also be recognized to raised scavenging chelating abilities of a combined treatments as compared to single treatment.

In the present study, antioxidant activity of 2-bromo-3-hydroxy-2-nitropropylcinnamate against alcohol induced oxidative damage in mammalian hepatocytes.

EXPERIMENTAL

Synthesis of 2-bromo-3-hydroxy-2-nitropropylcinnamate: 2-Bromo-2-nitropropan-1,3-diol (6 g, 0.02 mol) and cinnamoyl chloride (1.6 mL, 0.01 mol) have been dissolved in DMF (10 mL) using sulphuric acid (0.5 mL) as reagent. The mixture has been refluxed for approximately for 8 h and the reaction process has been checked by using TLC (hexane:ethyl acetate, 95:5). The reaction mixture has been poured into 50 mL distilled water and the organic component had been separated by separating funnel using chloroform as eluent. The solvent was treated with sodium sulphate (anhydrous) to remove traces of water present. The solvent was filtered and removed by rotavator. The column chromatography was utilized to purify compound by hexane:ethylacetate (99:1). Dark brown colour solid was obtained with a yield 2.26 g (68 %) having m.p. 116 ºC.

Animals: Wistar adult male albino rats weighed about 150-170 g were utilized. All the rats had been housed in the conven-tional lab situations as well as looked after on rat diet plan (Lipton India Ltd.) along with tap water *ad libitum* under an all-natural light dark cycle.

Ethics: The experimental animals used for this study were locally bred male Wistar rats from the experimental animal house of Faculty of Veterinary Medicine, Maharshi Dayanand University, Rohtak, India. The rats were managed as per recommendations of the Maharshi Dayanand University Animal Care and Use Research Ethics Committee. All protocols were appraised and approved by the committee with assigned number MDU-ACUREC/19/20/4433.

Optimum dose selection of 2-bromo-3-hydroxy-2-nitropropylcinnamate: Rats have been cateogrized into 5 groups of ten animals and given orally 20-100 mg/kg bw/day 2-bromo-3-hydroxy-2-nitropropylcinnamate with olive oil for 30 consecutive days. After 26 h of last dose delivery, the animals had been studied for mortality [19], behavioural toxicity or morbidity and then autopsied for examine the oxidative stress and hepatotoxicity. The optimum dose (20 mg) was used for the further experimentation of liver protection through antioxidant activity against alcohol induced oxidative damage.

Experimental design: After acclimatization, animals had been categroized into groups of 6 rats:

Group I: Untreated rats have been maintained on normal diet as well as served as control.

Group II: Rats were orally treated with 30% alcohol (3.0 mL/twice a day) for 30 days.

Group III: Rats received orally 2-bromo-3-hydroxy-2-nitropropylcinnamate (20 mg/kg bw/day in olive oil) for 30 days and 30% alcohol as in Group II.

Group IV: Rats received orally silymarin (20 mg/kg bw/day in olive oil) for 30 days and 30% alcohol as in Group II.

In end of the testing time, all the treated rats have been kept for next 26 h on starvation. Thereafter all rats have been anesthesized and by using cardiac puncture, blood samples have been collected along with the serum has been examined for several antioxidant markers like reduced glutathione [20],

vitamin C [21], vitamin E [22], ceruloplasmin [23] as well as β-carotene [24]. Liver was frozen at -20 ºC for the biochemical analysis of superoxide dismutage (SOD) [25], catalase (CAT) [26], glutathion peroxidase (GPx) [27], glutathione reductase (GR) [28] and gluthione-*s*-transferase (GST) [29]. At the same time, lipid peroxidation (LPO) [30] has been examined in liver as well as serum indicating formation of unsaturated fatty acid the hepatic cells.

Statistical analysis: Each and every values were expressed by mean ± SEM. All data have been examined by utilizing Student 't' test. The $p \leq 0.05$ a probability values have been noted.

RESULTS AND DISCUSSION

Results obtained from biochemical parameters showed that an administration of 30% alcohol to rats caused major peroxidative damage ($p \leq 0.001$) as showed by enzymatic and non-enzymatic antioxidants and lipid peroxidation by serum along with liver contents as shown in Tables 1 and 2. Rats treated with 30% alcohol showed a major ($p \leq 0.001$) altitude of lipid peroxidation in contents of liver as well as serum (Tables 1 and 2). In contrast, treatment with 2-bromo-3-hydroxy-2-nitropropylcinnamate (20 mg/kg) indicated a significant sinking effect on 30% alcohol induced rise of LPO in liver and serum contents (Tables 1 and 2). Table-1 represents that the hepatic antioxidants activities like CAT, SOD, GR, GST and GPx were decreased considerably ($p \leq 0.001$) up to 30% alcohol administration to the rats (Group II) if equated with Group I. These decreased hepatic antioxidant markers were significantly brought towards normalization by 2-bromo-3-hydroxy-2-nitropropylcinnamate in Groups III and IV.

The activities of GSH, vitamin C, vitamin E, β-carotene as well as ceruloplasmin in serum had been reduced in rats treated with 30% alcohol in Group II (Table-2). Simultaneous treatment with 2-bromo-3-hydroxy-2-nitropropylcinnamate afforded a substantial protection against 30% alcohol-induced decrease in the levels of serum antioxidants in Group III and silymarin treated Group IV (Table-2).

Oxidative damage was concerned in the many pathology diseases as well as situations together with cardiovascular diseases, diabetes, aging, caring along with inflammatory conditions. The antioxidants might offer conflict in contradiction of oxidative damage by inhibiting the lipid peroxidation, scavenging of the free radicals as well as various mechanisms [31]. The ROS formation the oxidative damage along with hepatocellular injury was associated to alcoholic liver disease. The observation suggested that Kupffer cells plays major roe for

TABLE-1
ANTIOXIDANT ACTIVITY OF BNPC AGAINST ALCOHOL-INDUCED OXIDATIVE DAMAGE THROUGH HEPATIC PARAMETERS

Groups	SOD mole (mg protein)	CAT (mol H_2O_2 consumed/min/ mg protein)	GPx (n mole NADPH consumed/min/mg protein)	GR (n mole NADPH consumed/min/ mg protein)	GST (mole CDNB-GSH conjugate formed/min/mg protein)	LPO (n mole MDA/mg protein)
Normal (vehicle treated) (Group I)	10.25 ± 0.55	61.33 ± 2.10	12.18 ± 0.48	16.47 ± 0.56	6.86 ± 0.34	1.88 ± 0.13
30% alcohol (3.0 mL/day, orally) (Group II)	4.15 ± 0.08[a]	23.29 ± 1.08[a]	4.76 ± 0.22[a]	7.31 ± 0.17[a]	2.55 ± 0.08[a]	4.42 ± 0.16[a]
30% alcohol + BNPC (20 mg/kg b.wt. day, orally) (Group III)	6.30 ± 0.09[a]	36.21 ± .1.10[a]	7.25 ± 0.19[a]	10.51 ± 0.18[a]	4.21 ± 0.10[a]	3.55 ± 0.24[c]
30% Alcohol + Silymarin (20 mg/kg b.wt. day, orally) (Group IV)	7.50 ± 0.22[a]	45.35 ± 1.15[a]	9.20 ± 0.31[a]	12.13 ± 0.12[a]	4.90 ± 0.18[a]	1

Levels of significance: Data are mean ± SEM (n = 6).
a = $p \leq 0.001$; Group II compared with control (Group I).
a = $p \leq 0.001$; c = $p \leq 0.05$; Group III and IV compared with Group II.

TABLE-2
ANTIOXIDANT ACTIVITY OF BNPC AGAINST ALCOHOL-INDUCED OXIDATIVE DAMAGE THROUGH SERUM PARAMETERS

Groups	GSH (mg/dL)	Ceruloplasmin (mg/dL)	β-Carotene (mg/dL)	Vitamin C (mg/mg protein)	Vitamin E (mg/mg protein)	LPO (n mole MDA/ mg protein)
Normal (vehicle treated) (Group I)	28.12 ± 1.78	15.25 ± 0.93	3.21 ± 0.17	2.56 ± 0.12	1.22 ± 0.09	2.39 ± 0.19
30% Alcohol (3.0 mL/day, orally) (Group II)	13.88 ± 0.99[a]	5.85 ± 0.14[a]	1.09 ± 0.13[a]	0.97 ± 0.07[a]	0.33 ± 0.02[a]	7.10 ± 0.88[a]
30% Alcohol + BNPC (20 mg/kg b.wt. day, orally) (Group III)	18.38 ± 0.27[b]	8.13 ± 0.17[a]	1.88 ± 0.15[b]	1.29 ± 0.11[c]	0.87 ± 0.06[a]	4.02 ± 0.21[b]
30% Alcohol + Silymarin (20 mg/kg b.wt. day, orally) (Group IV)	24.10 ± 0.78[a]	12.79 ± 0.37[a]	2.45 ± 0.18[a]	2.30 ± 0.19[a]	1.05 ± 0.07[a]	3.10 ± 0.29[a]

Levels of significance: Data are mean ± SEM (n = 6).
a = $p \leq 0.001$, Group II compared with control (Group I).
a = $p \leq 0.001$; b = 0.01; c = $p \leq 0.05$, Group III and IV compared with Group II.

ROS sources throughout the consumption of chronic alcohol, as well as are activated and primed to boost pro-inflammatory factors [32]. In addition, alcohol induced liver injury was accompanying having enlarged lipid peroxidation amount [33] and characterized by cirrhosis, fibrosis, necrosis, steatosis and inflammation [34].

The lipid peroxidation is found as a complex biochemical response that requires totally free radicals, oxygen, the metallic ions as well as a number of other things in the natural phone system. Simply because, lipids comprise nearly 60% of the parts inside biomembrans, the sole main perturbation is certain to be impacting the framework as well as functions of the cellular. Recently, research [35] reveals that lipids as well as the derivatives are recognized as important particles in the lipid and signal transduction peroxidation will be the emphasis of severe exploration in relation to the possible participation of its of illness as well as health.

In the current study, in serum along with liver the measurement of the lipid peroxidation is an appropriate technique for monitoring the damage of oxidative cell. The inhibition of raised up lipid peroxidation (LPO) has been witnessed in the 2-bromo-3-hydroxy-2-nitropropylcinnamate preserved groups owing its free radical as well as antioxidant scavenging activities by restabilising the biomembranes of hepatic parenchymal cells.

Antioxidant defence enzymes played a significant role to maintain physiological levels of hydrogen peroxide along with oxygen as well as eradicating peroxides produced from inadvertent exposure to drugs along with xenobiotics. The antioxidant compound might helpful to preserve health if it was taken continuously as the spices, drugs or dietary food [36]. The escalation in the antioxidant levels means SOD, GR, GPx, and CAT by 2-bromo-3-hydroxy-2-nitropropylcinnamate can be accredited to have biological importance in eradicating reactive free radicals which might have some impacts on ordinary function of mammalian hepatocytes. The activity of hepatic GST has been improved by 2-bromo-3-hydroxy-2-nitropropylcinnamate [27] raised the GST level through these compounds might have enabled the conjugation reaction of xenobiotic metabolism as well as might have improved the accessibility of non-critical nucleophiles for inactivation of the electrophiles, thus played a significant part in hepatic antioxidant system.

The current research work also shown that chronic exposure to alcohol significantly reduced the activities of non-enzymatic antioxidants namely vitamins E and C, ceruloplasmin, β-carotene and GSH in serum and this could be liable for hepatocellular injury existing. The supplementation of 2-bromo-3-hydroxy-2-nitropropylcinnamate magnified non-enzyematic antioxidants greatly in 30% alcohol medicated rats. GSH, vitamins E and C occur in the interconvertible kinds of theirs and also take part in the cleansing of poisonous reactive oxygen species. Regrowth to the reduced types of theirs is because of decreased glutathione. The spreading GSH mimics enhanced utilization to hole the no cost radicals [37]. Vitamin C is well-thoughtout being the best crucial antioxidant in additional cellular fluids [38] that is likewise safeguarding membranes against peroxidation by raising the task of β-tocopherol that will be the chief lipid soluble and also chain busting antioxidant.

Conclusion

The biochemical variations witnessed in hepatic damage appears to be mostly because of mechanism of oxyradical-mediated, which involves lipid peroxidation having given conditions of decreased levels of antioxidant, which scavenge lipid peroxides, superoxide and hydrogen peroxide. The obtained results suggested that 2-bromo-3-hydroxy-2-nitropropylcinnamate is effective in their antioxidant properties.

REFERENCES

1. E.B. Kurutas, The Importance of Antioxidants which Play the Role in Cellular Response against Oxidative/Nitrosative Stress: Current State, *Nutr. J*., **15**, 71 (2015); https://doi.org/10.1186/s12937-016-0186-5
2. D.-P. Xu, Y. Li, X. Meng, T. Zhou, Y. Zhou, J. Zheng, J.-J. Zhang and H.-B. Li, Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources, *Int. J. Mol. Sci*., **18**, 96 (2017); https://doi.org/10.3390/ijms18010096
3. H. Sies, Oxidative Stress: Oxidants and Antioxidants, *Exp. Physiol*., **82**, 291 (2012); https://doi.org/10.1113/expphysiol.1997.sp004024
4. S. Vertuani, A. Angusti and S. Manfredini, The Antioxidants and Pro-Antioxidants Network: An Overview, *Curr. Pharm. Des.*, **10**, 1677 (2004); https://doi.org/10.2174/1381612043384655
5. R.A. Miller and B.E. Britigan, Role of Oxidants in Microbial Pathophysiology, *Clin. Microbiol. Rev.*, **10**, 1 (1997); https://doi.org/10.1128/CMR.10.1.1
6. J. Chaudière and R. Ferrari-Iliou, Intracellular Antioxidants: From Chemical to Biochemical Mechanisms, *Food Chem. Toxicol.*, **37**, 949 (1999); https://doi.org/10.1016/S0278-6915(99)00090-3
7. H. Sies, Strategies of Antioxidant Defense, *Eur. J. Biochem.*, **215**, 2139 (1993); https://doi.org/10.1111/j.1432-1033.1993.tb18025.x
8. C.E. Cross, B. Halliwell, E.t. Borish, W.A. Pryor, B.N. Ames, R.L. Saul, J.M. McCord and D. Harman, Oxygen Radicals and Human Disease, *Annal Int. Med.*, **107**, 526 (1987); https://doi.org/10.7326/0003-4819-107-4-526
9. J.L. Marx, Oxygen Free Radicals Linked to Many Diseases, *Science*, **235**, 529 (1987); https://doi.org/10.1126/science.3810154
10. G.L. Plaa and H. Witschi, Chemicals, Drugs and Lipid Peroxidation, *Annu. Rev. Pharmacol. Toxicol.*, **16**, 125 (1976); https://doi.org/10.1146/annurev.pa.16.040176.001013
11. A.L. Cederbaum, Introduction: Role of Lipid Peroxidation and Oxidative Stress in Alcohol Toxicity, *Free Radic. Biol. Med.*, **7**, 537 (1989); https://doi.org/10.1016/0891-5849(89)90029-4
12. R.G. Thurman and J.A. Handler, New Perspectives in Catalase Dependent Ethanol Metabolism, *Drug Metab. Rev.*, **20**, 679 (1989); https://doi.org/10.3109/03602538909103570
13. H. Kleszczynska, D. Bonarska, H. Pruchnik, K. Bielecki, A. Piasecki, J. Luczynski and J. Sarapuk, Antioxidative Activity of New *N*-Oxides of Tertiary Amines: Membrane Model and Chromogen Studies, *Z. Naturforsch. C*, **60**, 567 (2005); https://doi.org/10.1515/znc-2005-7-809
14. J. Gabrielska, M. Soczyñska-Kordala and S. Przestalski, Antioxidative Effect of Kaempferol and Its Equimolar Mixture with Phenyltin Compounds on UV-Irradiated Liposome Membranes, *J. Agric. Food Chem.*, **53**, 76 (2005); https://doi.org/10.1021/jf0401120
15. J. Gabrielska, M. Soczyñska-Kordala, J. Hladyszowski, R. Zylka, J. Miskiewicz and S. Przestalski, Antioxidative Effect of Quercetin and Its Equimolar Mixtures with Phenyltin Compounds on Liposome Membranes, *J. Agric. Food Chem.*, **54**, 7735 (2006); https://doi.org/10.1021/jf060720a
16. R.F.V. de Souza and W.F. de Giovani, Antioxidant Properties of Complexes of Flavonoids with Metal Ions, *Redox Rep.*, **9**, 97 (1967).

17. N. Yamamoto, J.H. Moon, T. Tsushida, A. Nagao and T. Terao, Inhibitory Effect of Quercetin Metabolites and their Related Derivatives on Copper Ion-Induced Lipid Peroxidation in Human Low-Density Lipoprotein, *Arch. Biochem. Biophys.*, **372**, 347 (1999); https://doi.org/10.1006/abbi.1999.1516
18. W.Y. Boadi, P.A. Iyere and S.E. Adunyah, Effect of Quercetin and Genistein on Copper- and Iron-Induced Lipid Peroxidation in Methyl Linolenate, *J. Appl. Toxicol.*, **23**, 363 (2003); https://doi.org/10.1002/jat.933
19. W.J. Blot, J.Y. Li, P.R. Taylor, W. Guo, S.M. Dawsey and B. Li, The Linxian Trials: Mortality Rates by Vitamin-Mineral Intervention Group, *Am. J. Clin. Nutr.*, **62**, 1424S (1995); https://doi.org/10.1093/ajcn/62.6.1424S
20. M. Moron, J. Depierre and B. Mannervik, *Biochim. Biophys. Acta*, **582**, 67 (1979); https://doi.org/10.1016/0304-4165(79)90289-7
21. S.T. Omaye, J.D. Turnbull and H.E. Sauberlich, Selected Methods for the Determination of Ascorbic Acid in Animal Cells, Tissues and Fluids, *Methods Enzymol.*, **62**, 3 (1979); https://doi.org/10.1016/0076-6879(79)62181-X
22. H. Baker, O. Frank, B. Angelis and S. Feingold, Plasma Tocopherol in Man at Various Time Intervals after Ingesting Free or Acetylated Tocopherol, *Nutr. Rep. Int.*, **21**, 531 (1980).
23. H.A. Ravin, An Improved Colorimetric Enzymatic Assay of Ceruloplasmin, *J. Lab. Clin. Med.*, **58**, 161 (1961).
24. D.W. Bradley and C.L. Hornbeck, A Clinical Evaluation of an Improved TFA Micromethod for Plasma and Serum Vitamin A, *Biochem. Med.*, **7**, 78 (1973); https://doi.org/10.1016/0006-2944(73)90101-4
25. S. Marklund and G. Marklund, Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase, *Eur. J. Biochem.*, **47**, 469 (1974); https://doi.org/10.1111/j.1432-1033.1974.tb03714.x
26. H. Aebi, eds.: S.P. Colowick and N.O. Kaplan, Catalase *in vitro*, In: Methods in Enzymology, Vol. **5**, Academic Press: New York, vol. 5, pp 121-126 (1967).
27. D.E. Paglia and W.M. Velentine, Studies on the Quantitative and Qualitative Characterization of Erythrocyte Glutathione Peroxidase, *J. Lab. Clin. Med.*, **70**, 158 (1967).
28. I. Carlberg and B. Mennervick, Methods in Enzymology, vol. 113, Academic Press: New York, pp 484-490 (1985).
29. W.H. Habig, M.J. Pabst and W.B. Jacoby, Glutathione S-transferases. The First Enzymatic Step in Mercapturic Acid Formation, *J. Biol. Chem.*, **249**, 7130 (1974).
30. H. Ohkawa, N. Ohishi and K. Yagi, Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction, *Anal. Biochem.*, **95**, 351 (1979); https://doi.org/10.1016/0003-2697(79)90738-3
31. K.A. Youdim and J.A. Joseph, A Possible Emerging Role of Phyto-chemicals in Improving Age-Related Neurological Dysfunctions: A Multiplicity of Effects, *Free Radic. Biol. Med.*, **30**, 583 (2001); https://doi.org/10.1016/S0891-5849(00)00510-4
32. A.P. Bautista, Free Radicals, Chemokines, and Cell Injury in HIV-1 and SIV Infections and Alcoholic Hepatitis, *Free Radic. Biol. Med.*, **31**, 1527 (2001); https://doi.org/10.1016/S0891-5849(01)00745-6
33. A.A. Nanji, K. Jokelainen, G.L. Tipoe, A. Rahemtulla, P. Thomas, A.J. Dannenberg and A.E. Fisher, Curcumin Prevents Alcohol-Induced Liver Disease in Rats by Inhibiting the Expression of NF-κB-Dependent Genes, *Am. J. Physiol. Gastrointest. Liver Physiol.*, **284**, G321 (2003); https://doi.org/10.1152/ajpgi.00230.2002
34. H. Kono, G.E. Arteel, I. Rusyn, H. Sies and R.G. Thurman, Ebselen Prevents Early Alcohol-Induced Liver Injury in Rats, *Free Radic. Biol. Med.*, **30**, 403 (2001); https://doi.org/10.1016/S0891-5849(00)00490-1
35. R.A. Floyd, Role of Oxygen Free Radicals in Carcinogenesis and Brain Ischemia, *FASEB J.*, **4**, 2587 (1990); https://doi.org/10.1096/fasebj.4.9.2189775
36. R.P. Singh, B. Padmavathi and A.R. Rao, Modulatory Influence of Adhatoda Vesica (*Justicia adhatoda*) Leaf Extract on the Enzymes of Xenobiotic Metabolism, Antioxidant Status and Lipid Peroxidation in Mice, *Mol. Cell. Biochem.*, **213**, 99 (2000); https://doi.org/10.1023/A:1007182913931
37. A.T.A. Nandhini, S.D. Balakrishnan and C.V. Anuradha, Response of Liver Antioxidant System to Taurine in Rats Fed High Fructose Diet, *Indian J. Exp. Biol.*, **40**, 1016 (2002).
38. R. Stocker and B. Frei, ed.: H. Sies, Oxidative Stress: Oxidants and Antioxidants, Academic Press: London, pp. 213-243 (1991).