WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 7, 565-575. Research Article ISSN 2277 – 7105

ANTIOXIDANT STUDY AND PHENOLIC CONTENT OF *CARALLUMA FIMBRIATA* HERB

Abhinandan R. Patil*[1], Ajit B. Patil[1], Snehal S. Naingade[1], John I. Disouza[1]

Tatyasaheb Kore College of Pharmacy, Warnanagar-416113, M.S., India

Article Received on
11 July 2014,

Revised on 5 July 2014,
Accepted on 29 August 2014

***Correspondence for Author**
Dr. Abhinandan R. Patil
Tatyasaheb Kore College of Pharmacy, Warnanagar-416113, M.S., India

ABSTRACT

Antioxidant compound reduces the oxidative stress, which leading to reduce or neutralizing oxidation in cell. Antioxidant play major role in health protecting. The present study shows the total phenolic compound and antioxidant power of ethanolic and aqueous extract of *Caralluma Fibriata* herb. The total phenolic compound study done by Foline-Ciocalteu method and the antioxidant study done by DPPH assay, Hydrogen Peroxide assay, FRAP assay. The ethanolic extract of *Caralluma Fibriata* herb shows higher antioxidant property than aqueous extract.

KEY WORDS Total phenolic Compound, Antioxidant, *Caralluma Fibriata* herb.

INTRODUCTION

Antioxidants are chemicals that oppose or neutralize oxidation in cells. Antioxidants scavenge free radicals by inhibiting reactions within cells brought about by dioxygen or peroxide molecules, also called reactive oxygen species, or ROS, as well as by reactive nitrogen species. The total supply of antioxidants circulating in the body is the sum of antioxidant enzymes and acids manufactured by the body plus antioxidants consumed in foods [1]. Antioxidant compounds in food play an important role as a health-protecting factor. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk[2].During normal metabolism in aerobic cells, molecular oxygen is reduced to water; yet the stepwise transfer of electrons generates free reactive oxygen species (ROS),including superoxide radicals (O2-), hydrogen peroxide(H2O2), and hydroxyl radicals (OH). Other radicals suchas alkyl (R), alkoxyl (RO), and peroxyl (ROO) radicalsmay also be produced

endogenously, lipid peroxidationbeing another significant source of ROS[3]. Excessive generation of ROS and other radicals can damage proteins, carbohydrates, polyunsaturated fatty acids, and DNA,and may thus lead to oxidative stress and to a variety of degenerative processes and diseases such as aging, immunodeficiencies, neurologic disorders, inflammation, arteriosclerosis, coronary heart disease and certain cancers[4].

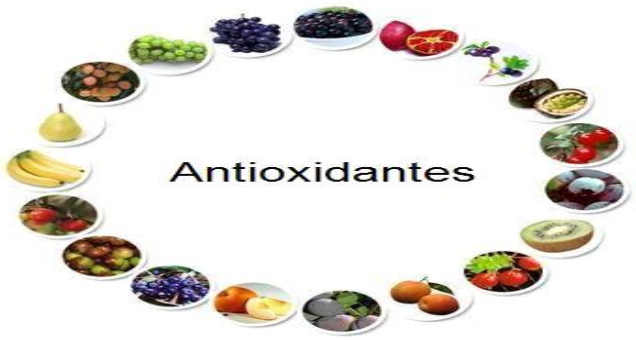

Fig.1. Antioxidant Foods

MECHANISM OF ANTIOXIDANTS

Hydrogen donation to free radicals by antioxidants. Formation of a complex between the lipid radical and the antioxidant radical (free radical acceptor).

Reaction of Antioxidants With Radicals:

R· + AH RH + A·

RO· + AH → ROH + A·

ROO· + AH → ROOH + A·

R· + A· → RA

RO· + A· → ROA

ROO· + A·→ ROOA

Antioxidant + O2 → Oxidized Antioxidant

Antioxidant Enzymes

Antioxidant enzymes play a fundamental role in maintaining the delicate redox balance in the body and are essential in keeping the physiological function andin coping with oxidant stress from endogenous or exogenous sources [5]

Herbs Extracts of many member s o f the Labiatae (Lamiaceae)fam-ily (ore gano, m arjoram, s avor y, sage, rosemar y, thyme, and b asil), which are antioxidative, have a h igh

total phenol content[6,7]. A number of herbs(chamomile, rosehip, hawthor n, and lemon verbena) can enhance the activity o f antioxidative enzymes such as superoxide dismutase and catalase in a dose-dependent manner and have been shown to enhance cell viability and provide protective effects against oxidative stress induced by hydrogen p eroxide[8]. *Caralluma Fimbriata* herb is a dry herb growing in the dry parts of the India. *Caralluma fimbriata herb*, a traditional Indian "famine food" with no history of adverse effects [9].The key phytochemical constituents of the herb are pregnane glycosides, flavone glycosides, megastigmane glycosides, and saponins. Some of the active components present in this plant are Caratuberside A, Caratuberside B, Bouceroside I-X, Tomenkogenin, Sitosterol etc[10]. Phenolic compounds exhibit a wide range of biological and physiological properties due to their ability to act as Antioxidants [11]. free radical scavengers 5,6 and chelators of divalent cations[12,13]. The antioxidant activity of phenol compounds is influenced by several factors, i.e. position and degree of hydroxylation, polarity, solubility, reducing potential, and stability of the phenoxy radical [14]. In this work, the antioxidant properties of the palm fruit extracts were studied by their ability to scavenge free radicals using the 2,2 diphenyl-1 picrylhydrazyl radical (DPPH.) reducing power and hydrogen peroxide scavenging assay.

The oxidative stress associated injury is a direct result of an imbalance between an increase in ROS production and a decrease in antioxidant reserve under various pathological processes Ischemic injury occurs when there is reduced blood supply or complete occlusion of an artery. The causes for ischemic insults vary from organ to organ, and rupture of atherosclerotic plagues with resultant formation of thrombi represents a major cause for acute ischemic injury in the heart, brain, lung, intestinal tract and other organs. Intermittent constriction or compression from the outside of vessels also causes a reduction or cessation of blood supply. Diabetic, Lung, heart and liver transplantation remains the only effective therapy for end-stage lung, heart or liver diseases[15] .The hyperglycemia-induced endothelial dysfunction that occurs in diabetes is not fully understood, but oxidative stress has been reported to play a key role in the initial insult. Multiple biochemical pathways that are known to increase the production of reactive oxygen species (ROS) have been linked to hyperglycemia/diabetes-induced vascular injury. These pathways include glucose auto-oxidation, the polyol pathway, and the formation of advanced glycation end products. The hyperglycemia-induced endothelial dysfunction that occurs in diabetes is not fully understood, but oxidative stress has been reported to play a key role in the initial insult. Multiple biochemical pathways that are known to increase the production of reactive oxygen

species (ROS) have been linked to hyperglycemia/diabetes-induced vascular injury. These pathways include glucose auto-oxidation, the polyol pathway, and the formation of advanced glycation end products[16]. The tissues exposed to oxidative stress in diabetes include renal and ocular tissues, which tend to suffer damage and cause some of the complications of diabetes [17].

MATERIALS AND METHODS

Chemicals and Instruments

DPPH, gallic acid, ascorbic acid, potassium ferricyanide, trichloroacetic acid, ferric chloride and phosphate buffered saline sodium carbonate, water was used for preparation of aqueous solutions

Absorbance measurements were recorded by a Shi-madzu UV-160A UV-Visible Reading Spectrophotometer using disposable cuvettes for visible range, and quartz cuvettes for measurements in the ultraviolet (UV) range.

Plant Material

Plant *Caralluma fimbriata herb* was collected from hilly region of Phanhala and Kundal. The specimen was authenticated at Shivaji university by Varsha jadhav. The plant leaves were shade dried and the dried leaves were made into a fine powder.

Preparation of Extract

Extraction is carried out by two methods by using soxhlet apparatus and maceration process. Extraction of ethanol is carried out by soxhlet apparatus where as maceration method is carried by dissolving One hundred grams of dry fine powder in 250 ml of water for 15 days. After filteration, solvent is removed.

Total Phenolic Compounds

Total phenolic content of dried extract was determined by foline-Ciocalteu method. A dilute concentration of extract (0.5 ml) was mixed with 0.5ml of 1:1 diluted folin-Ciocalteu reagent and 4ml of sodium carbonate (1M). The mixture were allowed to stand for 15 min and the total phenol content was determined by colourimeter at 765nm. A Standard curve was prepared by using an increasing concentration of Gallic acid in Methanol. A Standard curve was plotted using different concentration of Gallic acid (standard 0-1000 µg).Total phenolic compound was estimated as µg Gallic acid Equivalent (GAE)/mg of extract.[18]

Table.1. Total Phenolic Compounds of Ethanolic Extract of *Caralluma Fibriata* Herb.

Concentration(µg/ml)	*Caralluma fimbriata* Absorbance	Gallic acid bsorbance
50	75.06 ± 2.5	94.2 ± 0.5
100	72.13 ± 1.12	84.96 ± 2.9
200	65.5 ± 2.8	74.5 ± 1.1
500	59.7 ± 5.8	63.7 ± 0.80
1000	55.23± 6.5	52.03 ± 1.1

Values are Mean ± SEM of triplicate determination

ANTIOXIDANT MODELS

1. Antioxidant activity by 1, 1-diphenyl-1-picrylhydrazyl: Antioxidant activity of isolated compound was determined by 1,1-diphenyl-2 picryl-hydrazyl method [19]. Briefly, prepared 0.05 mM solution of DPPH in methanol and add 1.5 mL of this solution to 0.5 ml of isolated compound solution in methanol at different concentrations (100-300). Shake the mixture and allowed to stand for 30 min at room temperature. Absorbance was measured at 517 nm using a spectrophotometer. A blank reading without DPPH was used to remove the influence of the colour of the samples. A methanolic solution of DPPH was used as negative control. Ascorbic acid was used as a reference drug. All measures were carried out in triplicate. The DPPH radical scavenging activity was calculated using the formula.

$$\text{Percentage scavenging of DPPH radical} = \frac{100 \times (A_{\cdot} - A_s)}{A_{\cdot}}$$

Where, A_o is absorbance of the negative control and A_s is the absorbance of the sample.

Table.2. Free radical scavenging activity of *Caralluma Fimbriations* (CF) Ethanolic extract by DPPH radical inhibition.

CF Extract(µg/Ml)	% Inhibition	Ascorbic Acid g/Ml)	% Inhibition
50	12.73 ± 0.0375	50	29.29 ± 0.21
100	29.90 ± 0.0371	100	31.90 ± 0.05
150	37.28 ± 0.209	150	43.27 ± 0.18
200	42.78 ± 0.115	200	58.56 ± 0.28
250	57.89 ± 0.015	250	65.43 ± 0.19
300	63.73 ± 0.100	300	75.56 ± 0.13
IC50 value	185		170

Values are Mean ± SEM of triplicate determination

Table.3. Free Radical Scavenging Activity of *Caralluma Fimbriata* (CF) Aqueous Extract By DPPH Radical Inhibition.

CF Extract(µg/ml)	% Inhibition	Ascorbic acid (µg/ml)	% Inhibition
50	27.38 ± 0.13	50	25.6 ± 0.13
100	30.66 ± 0.10	100	31.78 ± 0.15
150	51.44 ± 0.20	150	41.74 ± 0.14
200	54.59 ± 0.24	200	50.49 ± 0.25
250	72.14 ± 0.15	250	65.48 ± 0.23
300	75.67 ± 0.06	300	68.29 ± 0.11
IC50 value	140		200

Values are Mean ± SEM of triplicate determination

1. Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing capability of is determined by the method of Oyaizu, 1986. In this assay, the color of the solution changes to various shades of green and blue, depending upon the reducing power of each antioxidant sample. The reducing capacity of compound may serve as significant indicator of its potential antioxidant activity. The presence of reductants such as antioxidant substance causes the reduction of the Fe3+ / ferricynide complex to the ferrous form. Therefore, the Fe2+ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm[20,21]

Different concentrations of sample (25-250 µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricynide (2.5 ml, 1%). The mixture was then centrifuged for 10 min at 1000 rpm. The upper layer of the solution (2.5 ml) is mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicates increased reducing power.

Reducing power assay (%) = [{Ao - A1/Ao}] ×100.

Where,
Ao is the absorbance of FeCl3,
A1 is the absorbance of FeCl3 solution in presence of the extract.

Table.4. Reducing Power Activity of *Caralluma Fimbriata* (CF) Ethanolic Extract

CF Extract(µg/ml)	% Inhibition	Ascorbic acid (µg/ml)	% Inhibition
50	18.43 ± 0.22	50	28.19 ± 0.09
100	28.16 ± 0.07	100	36,69 ± 0.14
150	34.04 ± 34.45	150	47.73 ± 0.07
200	43.55 ± 0.12	200	55.50 ± 0.14
250	54.79 ± 0.05	250	60.61 ± 0.06
300	65.55 ± 0.02	300	76.78 ± 0.11
IC50 value	230		220

Values are Mean ± SEM of triplicate determination

Table.5. Reducing Power Activity of *Caralluma Fimbriata* (CF) Aqueous Extract.

CFAq Extract(µg/ml)	% Inhibition	Ascorbic acid (µg/ml)	% Inhibition
50	46.42 ± 0.29	50	57.44 ± 0.23
100	52.65 ± 0.17	100	64.55 ± 0.13
150	57.67 ± 0.09	150	75.12 ± 0.22
200	58.68 ± 0.15	200	76.5 ± 0.25
250	60.16 ± 0.09	250	82.29 ± 0.16
300	73.38 ± 0.16	300	83.36 ± 0.37
IC50 value	72		52

Values are Mean ± SEM of triplicate determination

3. Antioxidant Activity By Hydrogen Peroxide Radical Scavenging Activity Hydrogen peroxide radical scavenging activity method [34]. In brief, the isolated compound 1 mL (50-250 µg mL^{-1}) solution was mixed with 2.4 mL, phosphate buffer (0.1 M, pH 7.4) and 0.6 mL of hydrogen peroxide solution (43 mM). After 10 min the absorbance was measured at 230 nm using spectrophotometer against a blank solution. The percentage inhibition was calculated. Each reading was performed in triplicate [22]

Table.6. Hydrogen Peroxide Scavenging Activity of *Caralluma Imbriata Herb* Ethanolic Extract.

CF Extract (µg/ml)	% Inhibition	Ascorbic acid (µg/ml)	% Inhibition
10	25.44 ± 0.22	10	80.51± 0.26
20	23.21 ± 0.14	20	82.40 ± 0.20
30	28.74 ± 0.08	30	85.46 ± 0.07
40	21.64 ± 0.17	40	85.8 ± 0.05
50	78.9 ± 0.07	50	87.26 ± 0.09
IC50 value	27		18

Values Are Mean ± SEM Of Triplicate Determination

Table.7. Hydrogen Peroxide Scavenging Activity of *Caralluma Fimbriata* Aqueous Extract.

CF Extract(µg/ml)	% Inhibition	Ascorbic acid (µg/ml)	% Inhibition
10	27.51± 0.22	10	87.56 ± 0.178
20	35.69 ± 0.19	20	88.39 ± 0.07
30	50.55 ± 0.14	30	89.61 ± 0.17
40	57.55 ± 0.11	40	90.38 ± 0.21
50	68.44 ± 0.20	50	91.51 ± 0.179
IC50 value	20		50

Values are Mean ± SEM of triplicate determination

RESULT AND DISSCUSSION

Total Phenolic Content of Extract

The total phenolic contents of the ethanol extracts were found to be 55.23 ± 6.5and 52.03 ± 1.1 of Gallic acid respectively. It is postulated that the treatment with ethanol may have resulted in the precipitation of non-phenolic compounds, and thus contributing to the higher Phenolic content in the ethanol extract.

Antioxidant Activity

The scavenging of the DPPH radical by hydrogen donating antioxidants (AH, Equation 1) is characterized by a rapid decline in the absorbance at 515 nm, followed by a slow step where the absorbance depreciates more gradually. The rapid reaction between antioxidants and DPPH**.** occurs with the transfer of the most labile H atoms to the radical, while the subsequent slow step depends on the residual H-donating capacity of antioxidant degradation products[23]

DPPH**.** + AH DPPH-H + A**.** *(Equation 1)*

The scavenging behavior of both aqueous and ethanol extracts are similar to that of Gallic acid, as seen from the disappearance of DPPH**.** Over time curves, Ascorbic acid, on the other hand exhibits a very rapid initial step (Fig. 2), and the disappearance of the purple colour of DPPH**.** Occurs almost immediately upon contact between reactants. These observations on the scavenging rates of Gallic and ascorbic acids are consistent with the observations reported by Sanchez-Moreno *et al.*,22[24].who classified these compounds as displaying, intermediate and rapid kinetic behaviour, respectively. A high antioxidant activity (greater than the standard) was found in the Ethanolic extract(IC50 185µg/mL). In ferric reducing assay, Aqueous as well Ethanolic extract of *Caralluma fimbriata herb* shows high antioxidant activity as compare to the satandard. Whereas, In Hydrogen peroxide assay Ethanolic extract shows significant IC50 value and Aqueous extract shows lower IC50 value. A lower IC50 value corresponds with a higher antioxidant power. These results indicate that all plant extracts have a noticeable effect on the scavenging of free radicals. This activity also increases with increasing concentration. Flavonoids, Glycosides, Saponine may be the compounds responsible for the antioxidant activity in these plants.

REFRENCES

1. Charles M. Benbrook Ph.D. Chief Scientis Elevating Antioxidant Levels in Food through Organic Farming and Food Processing, An Organic Center state of Science Review, Jan 2005.
2. Aruna Prakash, PhD, Fred Rigelhof and Eugene MIller, PhD, Antioxidant Activity, A publication of Medallion Labs
3. Simic MG, Bergtold DS, Karam LR (1989): Generation of oxy radicals in biosystems. Mutat Res 214: 3–12.
4. Gutteridge JMC (1993): Free radicals in disease processes:A compilation of cause and consequence. Free Radical Res Commum 19: 141–1583.)(Sies H (1991): Oxidative Stress: Introduction. London, Academic Press, 650.
5. Pasha K.V., Sadasivadu B., "Intracellular content of thiol compounds, thiobarbituric acid reactive substances and gamma-glutamy ltranspeptidase in rat brain during anoxia", NeurosciLett, 1984; 46:209-14
6. Chen Z. 2008. Research of antioxidative capacity in essential oils of plants. China Cond 11:40–3.
7. Chen ZY, Wang LY, Chan PT, Zhang Z , C hung HY, L iang C. 1998.Antioxidative a ctivity of g reen tea catechin extract compared with that of rosemar y extract. J Am Oil C hem S oc 75(9):11415.
8. Chen Z. 2008. Research of antioxidative capacity in essential oils of plants. China Cond 11:40–3.
9. Bansi Saboo, Dia care,Hemant Zaveri, Recent update in management of obesity and Overweight Patients: Standardized extract of Caralluma Fimbriata safe and effective Therapy. International Journal of Clinical Cases and Investigations 2011. Volume 2 (Issue 1), 5:9, 6th February 2011.
10. Scientific Opinion on the substantiation of a health claim related to ethanol-water extract of *Caralluma fimbriata herb* (Slimaluma and helps to reduce body weight pursuant to Article 13(5) of Regulation (EC) No 1924/20061 EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)
11. Catapano AL. Antioxidant effects of flavonoids. Angiology 1997; 48: 39-44.)4. Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, Riechel, TL. High-molecular weight plant phenolics (tannins) as biological antioxidants. J Agric Food Chem, 1998; 46:1887-1892.
12. Cavalini L, Bindoli A, Siliprandi N. Comparative evaluation of antiperoxidative action of silymarin and other flavonoids. Pharmacol Res Commun, 1978; 10: 133-*136.

13. Afanas'ev IB, Dorozhko AI, Brodskii AV, Kostyuk VA, Potapovitch AI. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. Biochem Pharmacol, 1989; 38: 1763-1769.
14. Decker EA. Antioxidant mechanisms. In: Akoh CC, Min DB, eds. Food lipids – chemistry, nutrition, and biotechnology. New York: Marcel Dekker, 1998; 397-421.
15. Behera S, Panigrahi R, Ramani Y, Babu S, Choudhury PK. Evaluation of Antioxidant Activity of Ocimum Canumhy droalcoholic Leaf Extract in the Prevention of Hepatic Ischaemia; 2012-Feb-16; WMC0030252012.
16. Ceriello A, Quatraro A, Giugliano D. New insights on non-enzymatic glycosylation may lead to therapeutic approaches for the prevention of diabetic complications. Diabet Med. 1992; 9:297-9. [PMID: 1576819], Collier A, Small M. The role of the polyol pathway in diabetes mellitus. Br J Hosp Med, 1991; 45:38-40.
17. Madsen-Bouterse SA, Kowluru RA. Oxidative stress and diabetic retinopathy: pathophysiological mechanisms and treatment perspectives. Rev Endocr Metab Disord. 2008; 9:315-27. [PMID: 18654858] ,Forbes JM, Coughlan MT, Cooper ME. Oxidative stress as a major culprit in kidney disease in diabetes. Diabetes. 2008; 57:1446-54. [PMID: 18511445]
18. Gao X, Ohlander M, Jeppsson N, Björk L, Trajkovski V.Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (Hippophae rhamnoides L.) during maturation. J Agric Food Chem 2000;48: 1485-1490.
19. Sreejayan N. and Rao MN. Free radical scavenging activity by curcuminoids. Arzneimittel forschung; 1996; 46: 169-171.
20. Chung YC, Chung CT, Chao WW, Lin CF, Chou ST. Antioxidant activity and safety of 50% ethanolic extract from red bean fermented by Bacillus subtilis IMR-NK1, J Agri Food Chem, 2002; 50: 2454-2458.
21. Mathew GM and Sashikumar JM. Antioxidant activity of Pseudarthria viscid. Indian J Pharma Sci, 2007; 69(4):581-582.
22. Sood S, Bansal SA, Muthuraman NS, Gill, Bali M. Therapeutic potential of *Citrus medica* L. peel extract in carrageenan induced inflammatory pain in rat. Res. J. Med. Plant; 2009; 3: 123-133
23. Dangles O, Fargeix G, Dufour C. Antioxidant properties of anthocyanins and tannins: a mechanistic investigation with catechin and the 3',4',7-trihydroxyflavylium ion. JChem Soc Perkin Trans 2000; 1653-1663. 21. Goupy P, Dufour C, Loonis M, Dangles O.

Quantitative kinetic analysis of hydrogen transfer reactions from dietary polyphenols to the DPPH Radical. J Agric Food Chem, 2003; 51: 615-62

24. Sánchez-Moreno C, Larrauri JA, Saura-Calixto F. A procedure to measure the antiradical efficiency of polyphenols.J Sci Food Agric, 1998; 76: 270-276.
25. Goldberg MD, Spooner J. Glutathione reductase. In: Methods of enzyme analysis, Eds. Bergmayer HU, Bergmayer J and Grabi M. Academic Press, Inc., Florida Vol. III, 3rd ed. 1983: 258-265.
26. Ricart-Jane D, Llobera M, Lopez-Tejero MD. Anticoagulants and other preanalytical factors interfere in plasma nitrate/nitrite quantification by the Griess method. Nitric Oxide, 2002; 6:178-185.
27. He RR, Yao XS, Li HY, Dai Y, Duan YH, Li YF, Kurihara H. The anti-stress effects of Sarcandra glabra extract on restraint-evoked immun compromise. Biol Pharm Bull, 2009; 32:247-252
28. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem, 1979; 95: 351-358.
29. Rao KS, Recknagel RO. Early onset of lipid peroxidation in rat liver after carbon tetrachloride administration. Exp. Mol. Pathol, 1968; 9: 271- 278.
30. Jiang ZY, Hunt JV, Wolf SP. Detection of lipid hydroperoxides using Fox method. Anal. Biochem, 1992 ;202: 384-389