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Evaluation of Ketoprofen Effect on Oxidative Stressed Mice

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ABSTRACT

Ketoprofen one of the propionic acid classes of Nonsteroidal Anti-Inflammatory Drugs (NSAID) with analgesic and antipyretic effects. It acts by inhibiting the body's production of prostaglandin it was patented in 1967 and approved for medical use in 1980. Ketoprofen is widely used for the treatment of pains, fever and inflammation is generally prescribed for arthritis related inflammatory pains or severe toothaches that result in the inflammation of the gums. Ketoprofen topical patches are being used for treatment of musculoskeletal pain. This study designed to evaluate if Ketoprofen has an antioxidant effect, relative to its analgesic, antipyretic and anti-inflammatory activities. Oxidative stress was induced by intraperetoneal injection of Hydrogen Peroxide (H_2O_2), and then a comparative study is made concerning the activities of the antioxidant enzymes Catalase (CAT), Superoxide Dismutase (SOD), Gluthation Reductase (GR), Succinate Dehydrogenase (SDH), and index of lipid peroxidation: Thiobarbituriquc Acid Reactive Substances (TBARS); compared to the group treated by L-ascorbic acid star of antioxidant and group ascorbic acid+H_2O_2, These results are statistically significant to conclude that NSAID drug Ketoprofen showed a strong antioxidant effect and protective effect against oxidative stress induced by H_2O_2 in comparison with the effect of ascorbic acid (vitamin C).

Keywords: Ketoprofen; Oxidative stress; Antioxidant; Anti-inflammatory; Hydrogen peroxide (H_2O_2)

INTRODUCTION

Ketoprofen is a Nonsteroidal Anti-inflammatory Drug (NSAID) belonging to the group of substituted 2-phenylpropionic acids. Its structural formula 2-(3-benzolpheny1)-propionic acid. Ketoprofen was synthesized by Rhone Poulenc research laboratories, Paris, in 1967 and was first approved for clinical use in France and the United Kingdom in 1973. The drug is currently marketed throughout the world in a variety of forms: capsules, injectable solutions, suppositories, and a topical gel. A controlled release capsule for once daily administration (Oruvail) was introduced in the United Kingdom. Extensive testing in the United States, confirming foreign clinical experience, demonstrated that ketoprofen is effective in treatment of arthritis. Furthermore, the drug has a well defined safety profile that offers significant advantages over aspirin in controlled studies. United States approval of clinical use of ketoprofen capsules in osteoarthritis and rheumatoid arthritis was granted in January 1986. Ketoprofen displayed potent activity against acute inflammation (increased vascular permeability, edema, and erythema), subacute inflammation (pleurisy, abscess, and granuloma formation), and chronic inflammation (experimental arthritis and synovitis) [1-8]. These tests showed ketoprofen to be 20 times more potent than ibuprofen, 80 times more potent than phenylbutazone, and 160 times more potent than aspirin in

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reducing inflammation from carrageenan induced abscesses in mice. The drug's potency was generally equivalent to that of indomethacin in most models of significance, in mice adjuvant arthritis, the minimally effective dose age of ketoprofen (2.5 mg/kg) could be doubled to produce even greater efficacy (up to 70% inhibition), while a similar dosage increase with indomethacin resulted in 100% mortality. Therefore, new NSAIDs without these side effects have long been pursued. The major mechanism by which ketoprofen and other NSAIDs exert their anti-inflammatory activity is through the suppression of prostaglandin biosynthesis by inhibiting Cyclooxygenases (COXs). Prostaglandins are major mediators of inflammation response, but also play a cytoprotective role in maintaining GI health and homeostasis. COXs, which catalyze the synthesis of prostaglandins from arachidonic acid, have two major subtypes COX-1 and COX-2. Oxidative stress takes place when the balance between the antioxidant defenses and the generation of Reactive Oxygen Species (ROS) is tipped in favor of the latter. Thus, Hydrogen Peroxide (H_2O_2) is directly involved in the production of ROS due to his high redox level. If not maintained oxidative damage accumulates known as oxidative stress [9,10]. ROS are products of regular cell metabolism ($1O_2$: singlet oxygen, H_2O_2: Hydrogen Peroxide, (OH): Hydroxyl radical, (O_2): Anion superoxide). They participate in many cellular events including signal transduction and antibacterial defense [11]. They are also capable a large dose of oxidizing cellular proteins, nucleic acids and lipids [12]. They contribute to cellular aging, mutagenesis carcinogenesis Alzheimer's disease, atherosclerosis and coronary heart disease possibly through destabilization of membranes, DNA damage, and oxidation of low density lipoprotein [13-19]. To protect from these highly reactive intermediates, living organisms possess a defense system consisting of enzymatic and non-enzymatic antioxidants that scavenge them. It is well established that the most important antioxidant enzymes are Superoxide Dismutase (SOD), which ensures the disputation of Superoxide (O_2) into a molecule of O2 , Catalase (CAT) which catalyzes the decomposition of Hydrogen Peroxide (H_2O_2) to water and oxygen, Glutathione Peroxidase (GPX), which reduces both H_2O_2 and organic peroxides by a glutathione dependent reaction, and Glutathione Reductase (GR), which catalyzes the NADPH dependent regeneration of glutathione (GSH) from the oxidized form (GSSG) generated by GPX. SDH catalyzes the oxidation of succinate to fumarate with the reduction of ubiquinone to ubiquinol, this occurs in the inner mitochondrial membrane by coupling the two reactions together. The objective of the present research was to examine and test precisely antioxidant activity of NSAID ketoprofen and even the protective effect at long term of treatment in mice, compared and analyzed the activity of increasing stress markers that suits hydrogen peroxide by oxidation, and reduction of the activity of stress markers suitable for treatment by L-ascorbic acid, with the decrease of the activity markers stress in the groups treated by Ketoprofen.

MATERIALS AND METHODS

Experimental Section

Tests: The test concerned 66 males adult Swiss albino mice weighting 25-30 grams. They were acclimatized to laboratory conditions before the test and fed ad libitum. They were fasted 16 hours prior to the treatment. All experiments were in accordance with the guidelines provided by the CPCSEA. Animals were divided into 11 groups (n=6 per group) as it's resumed in **Table 1**. Ketoprofen, vitamin C (L-ascorbic acid) and H_2O_2 were daily administered by intraperitoneal injection during 30 days.

Table 1: Summary of groups treated with Ketoprofen, H_2O_2 and vitamin C.

Groups	Number of mice	Treatment	Dose
1	6	NaCl	0.90%
2	6	H_2O_2	10 mg/kg
3	6	ketoprofen	3 mg/kg
4	6	Ketoprofen+H_2O_2	3 mg/kg+1 g/kg
5	6	Vitamin C	2mg/kg
6	6	Vitamin C+H_2O_2	20 mg/kg+1 g/kg

Preparation of Tissues for Analytic Procedures

Livers were rapidly thawed and homogenized using a Potter homogenizer (Elvehjem), in 3 volumes of ice cold 10 mM HEPES, 1 mM EDTA, 0.25 M Sucrose and 10 mM 2-mercaptoethanol, pH 7.4. All procedures were performed at 4°C. Homogenates were centrifuged at 7000 x g for 15 mn at 4°C (sigma 2-16 K) and the resultant supernatants were aliquoted and stored at –20°C for later enzyme assays.

Biochemical Assays

All assays were conducted at 25°C using Jenway 6405 UV/Visible spectrophotometer (Thermo electron corporation, bromate 3).

Protein Assay

Protein content was measured according to the Bradford procedure by using Bovine Serum Albumin (BSA) as standard. Protein reagent was added to protein solutions. The absorbance was measured at 595 nm after 10-15 mni of incubation in the dark.

Catalase

The consumption of 7.5 mM H_2O_2 in 50 mM potassium phosphate buffer (pH 7) was monitored at 240 nm as indicated in Ischiropoulos H, et al. [15].

Glutathione Reductase

The assay of Di ilio, et al., was used. The assay mixture contained 0.5 mM oxidized glutathione, 1 mM EDTA, 0.1 mM NADPH and 50 mM potassium phosphate buffer (pH 7.4) and NADPH consumption was monitored at 340 nm.

Superoxide Dismutase

The enzyme was assayed according to Paulette, et al. 5 mM EDTA, 2.5 mM $MnCl_2$, 0.27 mM NADH, 3.9 mM 2-mercaptoethanol in 50 mM potassium phosphate buffer (pH 7), monitored at 340 nm. The decrease in absorbance is measured after the addition of NADH to 0.27 mM as final concentration.

Succinate Dehydrogenase

The enzyme was assayed according to King 100 mM potassium phosphate buffer (pH 7.4), 0.3 mM EDTA, 0.053 mM DCIP and 100 mg of protein. The mixture was pre incubated 10 min at 25°C before adding 50 ml of KCN-succinate (containing 3.25 mg/ml of KCN in 0.5 M succinate). The measure of activity was done at 625 nm.

Thiobarbituric Acid Reactive Substances

Lipid peroxidation was estimated by the formation of Thiobarbituric Acid Reactive Substances (TBARS) and quantified in terms of Malondialdehyde (MDA) equivalents according to the method described by Samokyszyn and Marnett 1 ml of samples was added to 1 ml solution (0.375% thiobarbituric acid and 15% trichloracetic acid in 0.25 M hydrochloric acid). The tubes were heated at 100°C during 15 min and they were cooled in the ice to stop the reaction. One then carries out a centrifugation with 1000 x g during 10 min. The reading of supernatant was made to 535 nm.

Enzyme activity expression

The specific activity of each enzyme was calculated using the following formula:

$AS=(\Delta Abs/mn \times 1000)/(\varepsilon \times (P) \times Ve)$

ΔAbs/mn: Absorbance variation/minute

ε (Extinction coefficient):

ε (H_2O_2)=40 $M^{-1}.cm^{-1}$, for CAT

ε (NADH)=6220 $M^{-1}.cm^{-1}$, for SOD and GR

ε (DCIP)=19100 $M^{-1}.cm^{-1}$, for SDH

ε (MDA-TBA complex)=153000 $mM^{-1}.cm^{-1}$, for MDA

(P): Protein concentration

Ve: Assay volume

Statistical Analysis

In each assay, all experimental values were expressed as mean ± standard error of mean and the statistical significance between treated and control groups were analyzed by ANOVA. Differences were considered significant at the level $p < 0.05$. The analysis was performed with XLSTAT Version 2014.2.02

RESULTS AND DISCUSSION

Monitoring of body weight in mice: As shown in **Figure 1**, the body weight of all treated mice showed variations significantly different from those of the control, and control of stress. Group treated with H_2O_2 show a significant weight loss showing a low activity during treatment period shows the presence of oxidative stress. Significant increase in weight of groups (K+H) and (C+H) versus control stress, shows that NSAID Ketoprofen and L-ascorbic acid has restored the imbalance between prooxidant balance caused by the effect of hydrogen peroxide and antioxidant defense systems that lies at the agency level, compared the results with L-ascorbic acid and Ketoprofen in terms of weight compared to control mice and the control of stress, shows the presence of the antioxidant effect of the drug.

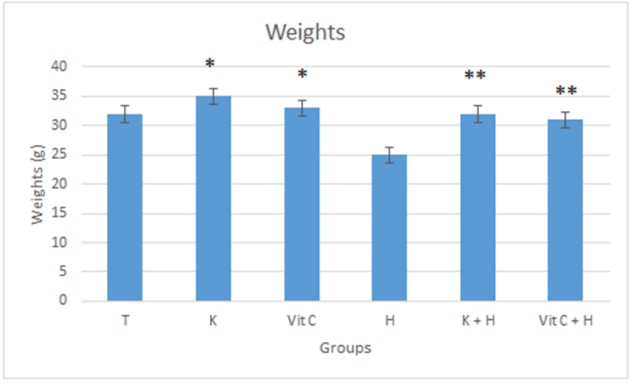

Figure 1: Weight gain in grams during 30 days of treatment.

T: Control; H: H_2O_2; K: Ketoprofen; K+H: Ketoprofen+H_2O_2; C: Vitamin C; C+H: Vitamin C+H_2O_2. *Significantly different from groups (T and H). **Significantly different from groups control of stress; the number of mice used in each group was 6.

The results in **Figures 2-6** showed that the activity of stress markers (CAT, SOD, GPx, SDH and TBARS) increased significantly in the treated group by hydrogen peroxide as compared with those of the control group. Explains presence of chronic oxidative stress in liver control stress group deduced by the presence of Reactive Oxygen Species (ROS) generate by oxidative power of the hydrogen peroxide.

Reactive Oxygen Species (ROS) produces oxidative stress which has been characterized in liver and includes, among several changes, an increase in the level of catalase, superoxide dismutase, glutathione peroxidase, succinate dehydrogenase and Thiobarbituric Acid Reactive Substances (TBARS), indicative of lipid peroxidation. The level of these enzyme activities in cells is crucial for determining the steady state levels of superoxide radicals and hydrogen peroxide, superoxide dismutase converts superoxide radicals to hydrogen peroxide, which is then decomposed by catalase, and succinate dehydrogenase catalyzes the oxidation of succinate to fumarate with the reduction of ubiquinone to ubiquinol. This occurs in the inner mitochondrial membrane by coupling the two reactions together. By one electron reductions of oxygen give rise to Reactive Oxygen Species (ROS) which include free as superoxide or Hydroxyl radical (OH) anion radicals. Other non-oxygen radical species can be produced, such as Hydrogen Peroxide (H_2O_2) and singlet oxygen the result of an imbalance between the balance of pro oxidants and antioxidant defense systems. As shown activity of stress markers (CAT, SOD, GPx, SDH and TBARS) decreases significantly in the treated group by (K+H) and (C+H) as compared to the group treated with hydrogen peroxide. Explains the restoration of balance redox reactions compared to antioxidant defense in the liver, by reducing significantly the production of Reactive Oxygen Species (ROS). also show significant protective effect against Reactive Oxygen Species (ROS) and against their oxidative effect on the molecular level, the significant decrease in the activity of stress marker in treated groups by K and C compared to the control groups showed a protective effect against Reactive Oxygen Species (ROS) by restoring the balance of redox reactions, this protection potential is mainly attributed to the antioxidant capacity in ascorbic acid to scavenge Reactive Oxygen Species (ROS), these results also show that Ketoprofen also has scavenging activity ROS, which explains the antioxidant effect of NSAID ketoprofen hide behind the other to already known. However, interpretation of these results becomes easy because administration of only of NSAID namely, ketoprofen in the absence of hydrogen peroxide decreases the amount of ROS in liver compared values of those recorded with hydrogen peroxide alone. The pro oxidant effects of hydrogen peroxide and the observed protective effect of Ketoprofen were explored by decreasing the activity of stress marker in treated groups (K+H) and (C+H) relative to control stress and from K and C compared to the control.

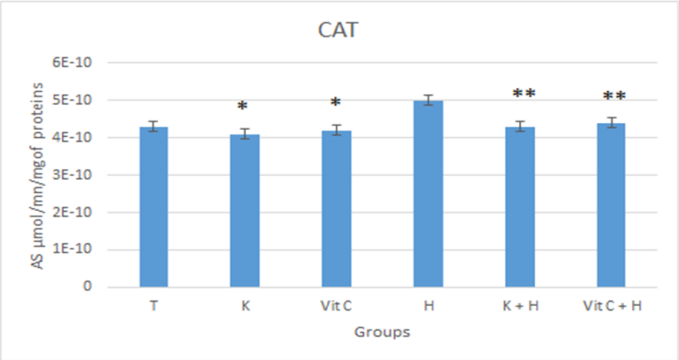

Figure 2: Evaluated antioxidant effect of NSAID ketoprofen by CAT activity.

T: Control; H: H_2O_2; K: Ketoprofen; K+H: Ketoprofen+H_2O_2; C: Vitamin C; C+H: Vitamin C+H_2O_2. *Signi icantly different from groups (T and H). **Signi icantly different from groups control of stress; the number of mice used in each group was 6.

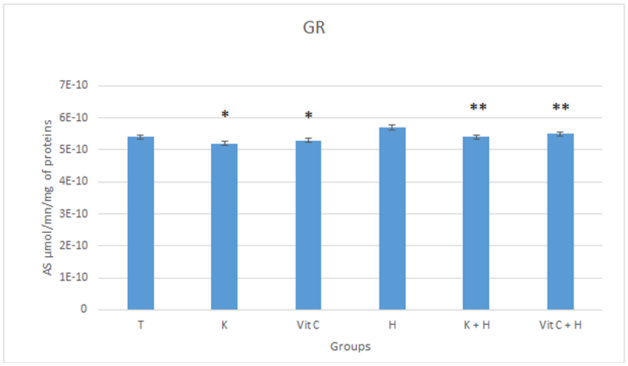

Figure 3: Evaluated antioxidant effect of nsaid ketoprofen evaluated by GR activity.

T: Control, H: H_2O_2; K: Ketoprofen; K+H: Ketoprofen+H_2O_2; C: Vitamin C; C+H: Vitamin C+H_2O_2. *Significantly different from groups (T and H). **Significantly different from groups control of stress; the number of mice used in each group was 6.

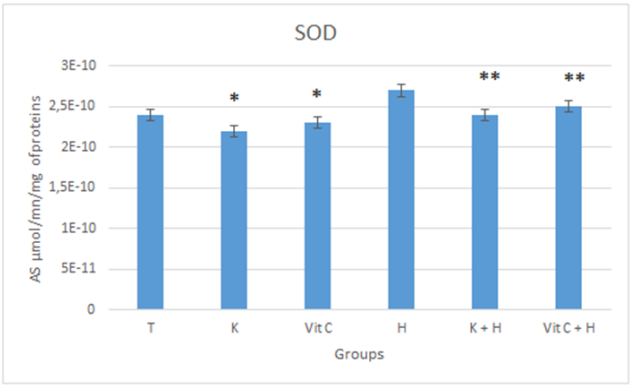

Figure 4: Evaluated antioxidant effect of NSAID ketoprofen evaluated by SOD activity.

T: Control; H: H_2O_2; K: Ketoprofen; K+H: Ketoprofen+H_2O_2; C: Vitamin C; C+H: Vitamin C+H_2O_2. *Significantly different from groups (T and H). **Significantly different from groups control of stress; the number of mice used in each group was 6.

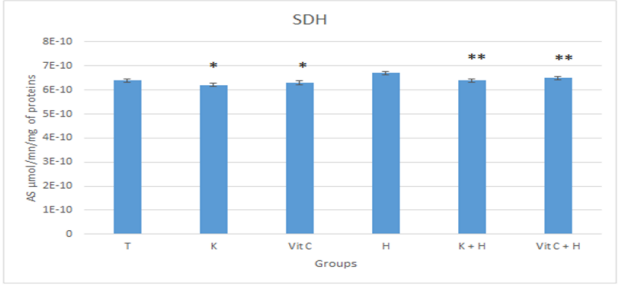

Figure 5: Evaluated antioxidant effect of Nsaid ketoprofen evaluated by SDH activity.

T: Control; H: H_2O_2; K: Ketoprofen; K+H: Ketoprofen +H_2O_2; C: Vitamin C; C+H: Vitamin C+H_2O_2. *Significantly different from groups (T and H). **Significantly different from groups control of stress; the number of mice used in each group was 6.

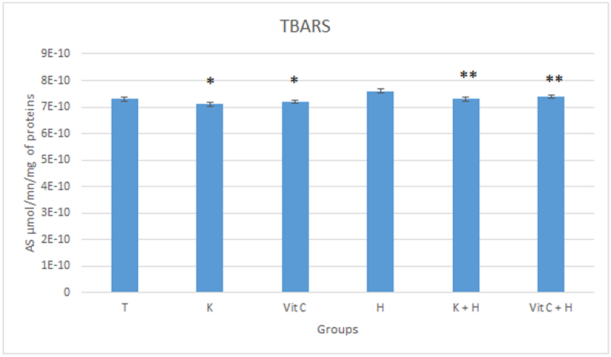

Figure 6: Evaluated antioxidant effect of nsaid ketoprofen evaluated by tbars activity.

T: Control; H: H_2O_2; K: Ketoprofen; K+H: Ketoprofen+H_2O_2; C: Vitamin C; C+H: Vitamin C+H_2O_2. *Significantly different from groups (T and H). **Significantly different from groups control of stress; the number of mice used in each group was 6.

CONCLUSION

This study has shown that Ketoprofen have antioxidant effect according to the results obtained from the treatment of mice with NSAID Ketoprofen by a dose (3 mg/kg) and treatment by a dose (3 mg/kg) in compared with stress control group treated by H_2O_2 by a dose (10 mg/kg) has demonstrated the important protective antioxidant effect of NSAID Ketoprofen against ROS compared to ascorbic acid effect. Have used vitamin C also known as ascorbic acid name, is definitely the star of vitamins to the general public. It lends many properties, some of which are not necessarily proven. The food industry also exploits the effects recognized antioxidants ascorbic acid form of derivatives used as preservatives (E300, E301 and E302). Vitamin C is involved in many body functions: It has antioxidant properties that help to help the body fight against the accumulation of heavy metals such as lead, mercury and cadmium. Moreover, the antioxidant activity of the ascorbic acid can neutralize free radicals, thereby protecting the cells of the organism aging and for strengthening the immune defenses. In addition, Ketoprofen has been demonstrated to inhibit chemically induced oxidative stress in animal. The protective effect of Ketoprofen against ROS has been presumably attributed to its ability to inhibit inflammation first and oxidation second. The anti-inflammatory and antioxidant action of Ketoprofen is believed to result from its non-specific inhibition of cyclooxygenase-2. Moreover, Ketoprofen has been reported to inhibit prostaglandin biosynthesis by inhibiting Cyclooxygenases (COXs) have two major subtypes COX-1 and COX-2, COX-2 is inducible and mediates inflammation response. In the state of inflammation there to increase the oxidation reaction in the body due to the activation of metabolism this explains the presence ROS in the state of inflammation. In addition, reactive oxygen species can also be produced by inflammatory cells as well as many other cellular sources. Metabolism of a variety of stressful chemical molecule also leads to generation of ROS. The main mode of action is irreversible inhibition of cyclooxygenase activity by acetylating serine in the active site of the enzyme. This causes the inhibition of prostaglandin and thromboxane synthesis. COX-2 is rapidly inducible by e.g. inflammatory stimuli and contributes to inflammatory responses, which explains the antioxidant effect of drug inhibition hide behind COX-2. We demonstrated a dose of Ketoprofen that inhibited oxidative power of hydrogen peroxide and production of reactive oxygen species is a (3 mg/kg). In conclusion, the results show clearly on the protective antioxidant effect of Ketoprofen against the intoxicate H_2O_2 and ROS generation, in order to strengthen the system of antioxidant defense and inhibited the activation pathways of the molecules responsible for causing oxidative stress.

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REFERENCES

1. Kantor TG (1986) Ketoprofen: A review of its pharmacologic and clinical properties. Pharmacotherapy. 6(3):93–103.
2. Mazieres B, Rouanet S, Guillon Y, Scarsi C, Reiner V (2005) Topical ketoprofen patch in the treatment of tendinitis: A randomized, double blind, placebo controlled study. J Rheumatol. 32 (8):1563–1570.
3. Mazieres B (2005) Topical Ketoprofen Patch. Drugs RD. 6(6):337–344.
4. Sekiya I, Morito T, Hara K, Yamazaki J, Ju YJ, et al. (2010) Ketoprofen Absorption by Muscle and Tendon after Topical or Oral Administration in Patients Undergoing Anterior Cruciate Ligament Reconstruction. AAPS Pharm SciTech. 11(1): 154–158.
5. Julou L, Guyonnet JC, Ducrot R, Fournel J, Pasquet J. Ketoprofen (1976) (2-(3-benzoylphenyl)-propionic acid). Main pharmacological properties-outline of toxicological and pharmacokinetic data. Scand J Rheumatol. 14:33-42.
6. Guyonnet JC, Julou L, Helsinki (1975) Symposium on ketoprofen. Rheumatol Rehabil. 1976;15(1):1-14.
7. Julou L, Guyonnet JC, Ducrot R, Pasquel J (1976) Some pharmacological and toxicological studies on ketoprofen. Eighth European rheumatology congress, Helsinki, 1975. Symposium on ketoprofen. Rheumatol Rehabil. 15(1): 5-10.
8. Perrone MG, Scilimati A, Simone L, Vitale P (2010) Selective COX-1 Inhibition: A Therapeutic Target to be Reconsidered. Curr Med Chem. 17(32):3769-3805.

9. Finkel T, Holbrook NJ (2000) Oxidants, oxidative stress and the biology of ageing. Nature. 408(6809):239-247.
10. Takabe W, Niki E, Uchida K, Yamada S, Satoh K, et al. (2001) Anti-atherogenic Antioxidants Regulate the Expression and Function of Proteasome α-Type Subunits in Human Endothelial Cells. Carcinogenesis. 276(44): 40497-40501.
11. Kawanishi S, Hiraku Y, Oikawa S (2001) Mechanism of guanine specific DNA damage by oxidative stress and its role in carcinogenesis and aging. Mutat Res, 488: 65-76.
12. Ischiropoulos H, Beckman JS (2003) Oxidative stress and nitration in neurodegeneration: Cause, effect, or association. J Clin Invest. 111(2):163-169.
13. Steinberg D (2002) Atherogenesis in perspective: Hypercholesterolemia and inflammation as partners in crime. Nat Med. 8(11):1211-1217.
14. Tian WN, Braunstein LD, Apse K, Pang J, Rose M, et al. (1999) Importance of glucose-6-phosphate dehydrogenase activity in cell death. Am J Physiol. 276:C1121-C1131.
15. Mora A, Paya M, Rios JL, Alcaraz MJ (1990) Structure activity relationships of polymethoxyflavones and other flavonoids as inhibitors of non-enzymic lipid peroxidation. Biochem Pharmacol. 40:793-797.
16. Takabe W, Niki E, Uchida K, Yamada S, Satoh K, et al. (2001) Acrolein oxidative stress promotes the development of transformation: involvement of a potent mutagenic lipid peroxidation product, acrolein. Carcinogenesis. 22(6):935-941.
17. Kondo K, Hirano R, Matsumoto A, Igarashi O, Itakura H (1996) Inhibition of LDL oxidation by cocoa. Lancet. 348:1514-1518.
18. Kirkman HN, Gaetani GF (1984) Catalase: a tetrameric enzyme with four tightly bound molecules of NADPH. Proc Natl Acad Sci USA. 1984, 81: 4343-4347.
19. Gaetani GF, Galiano S, Canepa L, Ferraris AM, Kirkman HN (1989) Catalase and glutathione peroxidase are equally active in detoxification of hydrogen peroxide in human erythrocytes. Blood. 73: 334-339.