NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS), FREE RADICALS AND REACTIVE OXYGEN SPECIES (ROS): A REVIEW OF LITERATURE

Karam-Allah S. Mahmood[1], Jawad H. Ahmed[2], Abdullah M. Jawad[3]

ABSTRACT

A wide controversy exists regarding the effect of NSAIDs on the oxidative status. Each member of these NSAIDs has been shown to act both as antioxidant or pro-oxidant in different test systems, using different concentrations and various oxidative stress-inducing agents. This review tackles this problem and tries to look for factors that might be responsible for this variation in results.

INTRODUCTION

The mechanism behind the great inter-individual variation in response to non-steroidal anti-inflammatory drugs (NSAIDs) and also behind their recent uses in chemoprevention of cancer is still poorly understood. One possible explanation might be through their effect on free radicals and reactive oxygen species and, thus, on the oxidative status.[1] Free radicals can be generated by oxidizing or reducing reactions. A free radical is defined as any species capable of independent existence that contains one or more unpaired electrons.[1] By this definition, oxygen which has two unpaired electrons is a free radical, whereas hydrogen peroxide (H_2O_2), which has no unpaired electrons, is not a free radical, but classified as reactive oxygen species (ROS).[2] Excessive reactive species can deplete the endogenous antioxidant system, giving rise to a condition known as oxidative stress. During the condition of oxidative stress, the generation of free radicals can have several consequences. Free radicals can react with and cause damage to DNA, lipids and proteins.[3] Examples of free radicals and ROS include: superoxide anion (O^{-}_2), hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2), nitric oxide and nitric dioxide, and peroxynitrite.[3]

Biological sources of free radicals and ROS

Many reactive species produced by biological system are intermediate products of several enzymatic and non-enzymatic reactions that are beneficial to the body. ROS and reactive nitrogen species (RNS) are formed during normal physiological processes that occur when the cell is not under stress.[4] The endogenous sources for ROS production in human and in animals may be as follows:[5]

- **Mitochondrial respiratory chain (O^{-}_2).**
- **Inflammation and phagocytosis (O^{-}_2, $OH^{\cdot}$, H_2O_2, HOCl).**
- **Xanthine oxidase (O^{-}_2).**
- **Vascular NAD(P)H oxidase (O^{-}_2).**
- **Cycloxygenase ($LOO^{\cdot}$).**
- **Free iron and copper as metal ions ($OH^{\cdot}$).**
- **Reaction between O^{-}_2 and NO to yield peroxinitrites.**
- **Reaction between H_2O_2 and peroxinitrites (singlet oxygen).**
- **Auto-oxidation of catecholamine.**
- **Ischemia/reperfusion (ROS and RNS).**
- **Prolonged severe emotional stress (ROS and RNS).**

Classification of antioxidant defences

Cells have complex antioxidant systems and chemical sequesters that help prevent oxidative damage caused by high free radical concentrations. These defence systems are classified as endogenous (e.g. Superoxide dismutase, Catalase, Glutathione peroxidase) and exogenous antioxidants (vitamin C, vitamin E, beta-carotenes and flavonoids).[6-8]

The role of metal ions in the induction of oxidative stress

Several metals, in their reduced forms, can catalyze the production of radicals from H_2O_2 via Fenton reaction. The transition metals of interest are those that have variable valence which allow them to undergo changes in oxidation state involving one electron. This is true for iron, cupper and manganese but not zinc which has only one valence which, unlike iron, cupper and manganese, prevents it from promoting radical reactions.[9-11] Fenton reaction occurs when a mixture of H_2O_2 and ferrous salt (Fe^{+2}) reacts with many organic molecules, as was first observed by Fenton in 1894. The reactivity is most likely due to formation of the hydroxyl radical ($OH\cdot$).[11]

[1]MSc(Pharmacology), Directorate of Basrah
[2]MBChB, PhD (UK), Assistant Professor, Department of Pharmacology, College of Medicine, University of Basrah, Iraq
[3]MBChB ,PhD (UK), Professor, Department of Pharmacology, College of Medicine, University of Basrah, Iraq.

Non-Steroidal Anti-inflammatory Drugs (NSAIDs) and Oxidative Stress

In the literature, it was surprising to find a great controversy on the effect of NSAIDs on the oxidative status. They, sometimes, act as oxidants and in other instances as antioxidants.

NSAIDs as antioxidants

There are many examples in which NSAIDs act as antioxidants at different clinical and laboratory settings. In one study, ibuprofen was tested on iron-mediated oxidation of LDL.[12] The authors had shown that the properties of ibuprofen are due both to inhibition of OH• generation in Fenton reaction and to the chelation of iron ions. Zapolska-Downar and Naruszewicz[13] found that ibuprofen exerted effects such as inhibition of adhesion and transendothelial migration of leukocytes, suppressing intracellular production of reactive oxygen species and oxidative modification of LDL. In a recent study, Dabhi et al[14] concluded that ibuprofen showed antihyperlipidemic, antiatherosclerotic, antioxidant, anti-inflammatory and non-ulcerogenic activity in atherosclerotic animals. Finally, Dokmeci et al[15] confirmed the protective effects of ibuprofen in the experimental testicular torsion/detorsion model in rats due to its anti-inflammatory and antioxidant effects.

Celecoxib, a COX-2 selective inhibitor, was shown to have the ability to inhibit lipid peroxidation (induced by ferrous salts in rat liver whole homogenate) and to scavenge hydroxyl radicals.[16] The exact mechanism by which celecoxib inhibited radical generation is not known. It may be due to direct scavenging of the peroxyl radicals or by donating reducing equivalents to the peroxyl radicals.[16] After oral administration, aspirin is rapidly converted to salicylic acid.[17] Salicylic acid can decrease oxidative stress and pro-inflammatory and potentially neoplastic prostaglandins with a concomitant increase in glutathione peroxidase activity. Salicylic acid may contribute to the protective effect of aspirin against colon cancer.[18] The suppressive and protective effects of ASA (acetyl salicylic acid) as an anti-cancer drug might, therefore, be partially ascribed to its antioxidant properties.[19-22] Acute ethanol intoxication produces widely studied hepatic metabolic disturbances, like limited gluconeogenesis, accumulation in triacylglycerides and reduction in protein synthesis.[23] Of practical interest is the finding that several NSAIDs administered to rats simultaneously with ethanol diminished some indicators of liver damage promoted by this hepatotoxic compound. Thus, the use of NSAIDs (aspirin, naproxen, nimesulide, and piroxicam) partially reversed the increase in triglycerides and thiobarbituric acid-reactant substances as well as the decrease in reduced and total glutathione produced by acute ethanol intoxication.[24-26] It has been shown that non-steroidal anti-inflammatory drugs (NSAIDs) (aspirin, ibuprofen and naproxen) appeared to delay the onset and slow the progression of Alzheimer's disease. NSAIDs are reported to neutralize the destructive hydroxyl free radicals. In the human body, this could prevent radical-mediated neurotoxic cell death and hinder the formation of neuritic plaques in the brain.[27] Antwerpen et al [28] found that oxicams are more reactive against reactive oxygen species than nimesulide and ibuprofen. This is not so pronounced for $OH^{\bullet}$ scavenging and the absence of interaction with H_2O_2 brings no further information concerning this reactive oxygen species. More interesting is the scavenging of HOCl since a substantial difference was observed between the oxicams and nimesulide. The importance of enol groups, inhibition of polymorphonuclear leukocyte migration, decreased oxygen radical production, and inhibition of lymphocyte function are suggested mechanisms.[29] Cyclooxygenase (COX) and lipoxygenase (LOX) pathways cause generation of reactive oxygen species (peroxides and superoxide anions) and peroxides, themselves, can trigger COX and LOX activity.[30] Prostaglandins are also involved in the activation of NADP(H) oxidase in neutrophils.[31] The results of Chakraborty et al[32] indicated that diclofenac sodium, ibuprofen, flurbiprofen, paracetamol (although it is an analgesic but not a NSAID), nimesulide and celecoxib exerted mild antioxidant activity.

In the study of Kadiiskaa et al [33], after induction of oxidant stress in rats with CCl4, lipid peroxidation products measured in plasma and urine demonstrated that isoprostanes and MDA can be partially inhibited to different extents by cyclooxygenase inhibitors

(indometacin and meclofenac). The lowering of isoprostane and MDA formation may be the result of the suppression of nonenzymatic lipid peroxidation rather than diminution of catalytic generation of isoprostanes or MDA by the cyclooxygenases. Fernandes et al[34] pointed out that several NSAIDs, including indomethacin, tolmetin, ketorolac, acemetacin and oxaprozin can effectively inhibit the peroxyl-radical derived from 2, 2′-azobis (2-amidinopropane) dihydrochloride in various noncellular *in vitro* systems. The order of the scavenging activity is acemetacin = tolmetin > ketorolac > indometacin > oxaprozin. While Parij et al[35] found that these NSAIDs possessed no pro-oxidant activity. Nevertheless, it has been suggested that the anti-inflammatory activity of NSAIDs may also be partly due to their ability to scavenge ROS and RNS and to inhibit the respiratory burst of neutrophils triggered by various activator agents.[34] Oktar et al[36] found that burn-induced intestinal and liver oxidative damage in rats using GSH and MPO activity as markers can be prevented by ketorolac, nimesulide and piroxicam. On the other hand, Kalapatapu et al [37] proved that ketorolac can attenuate the oxidative stress (measured by MDA and GSH) in postoperative intimal hyperplasia in a rat carotid endarterectomy. Diclofenac, indomethacin ibuprofen, naproxen and paracetamol at the therapeutic and higher concentrations, and dipyrone at high concentration exerted a statistically significant inhibition of H_2O_2-forced erythrocytic membrane lipid peroxidation.[38] Kamiyama et al[39] showed that acetaminophen did not increase diene conjugates *in vivo* and it inhibited thiobarbituric acid reactive substance production *in vitro*.

NSAIDs as pro-oxidants

NSAIDs are known to produce various tissue damage such as gastro-intestinal damage, nephrotoxicity, and hepatotoxicity. There is evidence that oxidative stress is involved in these toxicities.[40-42] The mechanism by which NSAIDs cause injury to the gastric mucosa is mainly due to the inhibition of cyclooxygenase enzyme (COX) and suppression of prostaglandin-mediated effects on mucosal protection.[43] It has also been proposed that neutrophil and oxygen radical-dependent microvascular injuries may be important processes that lead to mucosal damage in response to administratioin of NSAIDs (ASA, diclofenac, indometacin, paracetamol and piroxicam).[40] Other hypotheses that might explain the mechanism of NSAID-induced toxicity in the small intestine involves the action of these drugs in uncoupling or inhibiting oxidative phosphorylation.[44] Basivireddy et al [45] had shown that acute indometacin poisoning produced free radical-induced damage in enterocytes, with the villous tip cells being particularly susceptible to these effects. These effects appeared to be mediated through the generation of oxygen free radicals. Increased production of these reactive molecules may result from the mitochondrial dysfunction produced by indomethacin,[46-47] infiltration of neutrophils into the mucosa in response to this drug,[48] increased activity of xanthine oxidase [49] or a combination of these factors. Long term use of low dose aspirin (ASA) has been recommended to reduce the risk of heart attack but gastric bleeding and formation of gastrointestinal ulcers are the most common adverse effects. It has been shown that, ASA damages gastric mucosa by inhibiting the synthesis of protective prostaglandins and by having direct action on the mucosa. This results in enhancing acid back-diffusion and microvascular injury accompanied by the activation of neutrophils that produce excessive oxygen-derived free radicals which cause lipid peroxidation and tissue damage. [50-51] Exposure of low-density lipoprotein (LDL) preparations to meyloperoxidase enzyme in presence of salicylates revealed that the drug could act as a catalyst of lipid oxidation in LDL .[52] In the study of Nair et al, [53] the effects of two cycloxygenase-2 (COX-2) selective inhibitors, celecoxib and nimesulide as compared to a non-selective COX inhibitor, aspirin, were studied in rat intestine. The study showed a significant increase in the lipid peroxide levels as TBA-reactive substances as well as the conjugated dienes, except for celecoxib treated group which showed a decrease. Ibuprofen-induced damage to gastric mucosa after oral administration was accompanied by a significant increment in myeloperoxidase (MPO) activity (as an index of neutrophil activation), as well as lipid peroxidation (LP) levels and xanthine oxidase

(XO) activity.[54] Consequently, it is concluded that the active oxygen species, derived both from XO and activated neutrophils, could play a role in the pathogenesis of gastric injury induced by ibuprofen. On the other hand, celecoxib and indomethacin both exerted a statistically significant increase of MDA content, representing a significant peroxidation.[32] Browne et al[55] investigated the pro-oxidant properties of ketorolac after evaluation of both beta-oxidation of palmitate and oxidative phosphorylation in rat hepatic mitochondria. The anti-ulcer effect of melatonin on gastric lesions caused by piroxicam was studied by Bandyopadhyay et al.[56] Increased lipid peroxidation, augmented protein oxidation and decreased glutathione content of the gastric tissue following piroxicam treatment indicated a possible involvement of oxidative stress in this NSAID-induced gastropathy.[56] Indomethacin can induce nephrotoxicity. Inhibition of PG is involved in this toxicity. In addition, inhibition of oxidative phosphorylation that can lead to a decrease in ATP production,[57] and production of ROS, resulting in oxidative stress, had also been seen as possible mechanisms in indometacin or diclofenac-induced kidney damage.[58] It has been reported that diclofenac-induced hepatotoxicity in rats was associated with the production of reactive oxygen species (ROS).[59] Diclofenac, however, induces liver damage through various mechanisms such as mitochondrial permeability transition,[60] activation of cytochrome P450[61] in addition to generation of ROS. [61-62] A number of *in vitro* animal models have been used to investigate the possible mechanisms of NSAID-related hepatotoxicity. Studies using rat liver mitochondria and freshly isolated rat hepatocytes showed that diphenylamine, which is common in the structure of NSAIDs, uncouples oxidative phosphorylation, decreases hepatic ATP content and induces hepatocyte injury.[63-64] Furthermore, diclofenac is more toxic to drug-metabolizing than to non-metabolizing cell lines.[63] The *in vitro* cytotoxicity correlated well with the formation of 5-hydroxydiclofenac and particularly with the 5-dihydroxydiclofenac metabolite; the latter in particular can inhibit ATP synthesis.[63] Mitochondrial permeability transition has also been shown to be important in diclofenac-induced liver injury, resulting in generation of reactive oxygen species, mitochondrial swelling and oxidation of NADPH and protein thiols.[64] Overdose of paracetamol causes hepatotoxicity, which can in severe cases lead to liver failure in experimental animals and humans.[65] Although a large percentage of the dose of paracetamol is directly conjugated with glucuronic acid or sulfate, a significant amount of paracetamol is metabolized by the cytochrome P450 system.[66] This leads to the formation of a reactive metabolite, presumably N-acetyl-p-benzoquinoneimine (NAPQI), which reacts rapidly with glutathione (GSH).[66] Thus, paracetamol metabolism causes dramatic depletion of cellular glutathione levels in the liver. If the formation of the reactive metabolite exceeds the capacity of hepatocellular glutathione, NAPQI will covalently bind to cellular proteins.[67] Paracetamol challenge (300 mg/kg, i.p) for 7 days caused a significant increase in the levels of bilirubin, liver enzymes, TBARS, and iron, while catalase activity and total protein level were reduced significantly in the serum and liver homogenate.[68] Naproxen-induced lipid peroxidation in the liver microsomes and the isolated hepatocytes of rats, was found to be caused by the reactive oxygen species produced during naproxen oxidative metabolism but not by naproxen or its metabolites.[69-71] Interestingly, ferrous ions were released from the liver microsomes which underwent lipid peroxidation by naproxen.[72] The intracellular glutathione (GSH) decreased and the oxidized glutathione and glutathione disulfide (GSSG) increased during naproxen metabolism in the isolated rat hepatocytes.[73] It has been shown that ferrous ion released from rat liver microsomes contributes to naproxen-induced microsomal lipid peroxidation.[72] In conclusion, this controversy (summarized in Table-1) might be attributed to the different biological systems used to test the effect of these drugs, different concentrations utilized, or to the level of induction of the oxidative status. Further studies taking these factors into consideration are worth performing.

Table 1. Summary of the controversy on the effect of NSAIDs on the oxidant status

	NSAIDS AS ANTIOXIDANTS	NSAIDS AS PRO-OXIDANTS
Aspirin (Acetylsalicylic acid)	Janice et al (2006)[18] Hsu and Li (2002)[19] Zentella de Pina et al (1992),(1993) and (1994)[24-26] Basu et al (2001)[27] Muller-Peddindhaus and Muel (1987)[74] Wasil et al (1987)[76]	Wallace and Granger (1992)[40] Mc Carthy et al (1995)[50] Fossiline et al (1998)[51] Hermann et al (1999)[52] Nair et al (1990)[75]
Ibuprofen	Kennedy et al (1990)[12] Zapolska-Downar and Naruszewicz (2001)[13] Dabhi et al (2008)[14] Dokmeci et al (2007)[15] Basu et al (2001)[27] Antewrpen et al (2004)[28] Chakraborty et al (2007)[32] Orhan and Sahyyn (2001)[38] Muller-Peddindhaus and Muel (1987)[74]	Balasubramanian et al (2004)[77] Jiménez et al (2004)[78]
Naproxen	Zentella de Pina et al (1992), (1993) and (1994)[24-26] Basu et al (2001)[27] Orhan and Sahyyn (2001)[38] Muller-Peddindhaus and Muel (1987)[74]	Yokoyama et al (1994), (1995) and (1998)[70,71,73] Ji et al (2001)[72] Ishiyama (1990)[79]
Diclofenac	Chakraborty et al (2007)[32] Orhan and sahyyn (2001)[38] Muller-Peddindhaus and Muel (1987)[74]	Wallace and Granger (1992)[40] Masubushi et al (2000) [64]
Indometacin	Kadiiskaa et al (2005)[33] Fernandes et al (2004)[34] Orhan and sahyyn (2001)[38] Muller-Peddindhaus and Muel (1987)[74]	Chakraborty et al (2007)[32] Wallace and Granger (1992)[40] Hickey et al (2001)[41] Basiviredy et al (2002)[45] Mingatto et al (1996)[57]
Paracetamol	Chakraborty et al (2007)[32] Orhan and sahyyn (2001)[38] Kamiyama et al (1993)[39] Muller-Peddindhaus and Muel (1987)[74]	Wallace and Granger (1992)[40] Nelson (1990)[66] Ajith et al (2007)[68]
Celecoxib	Chang et al (2004)[16] Nair et al (1990)[75]	Chakraborty et al (2007)[32]
Nimesulide	Zentella de Pina et al (1992), (1993) and (1994)[24-26] Antewrpen et al (2004)[28] Chakraborty et al (2007)[32] Oktar et al (2002)[36]	Nair et al (1990)[75]
Piroxicam	Zentella de Pina et al (1992),(1993) and (1994)[24-26] Antewrpen et al (2004)[28] Oktar et al (2002)[36] Orhan and Sahyyn (2001)[38] Muller-Peddindhaus and Muel (1987)[74]	Furst and Tino Muster (2001)[29] Van Der Vliet and Bast (1992)[30] Dana et al (1994)[31] Wallace and Granger (1992)[40] Bandyopadhay et al (2004)[56]
Ketorolac	Fernandes et al (2004)[34] Oktar et al (2002)[36] Kalapatapu et al (2007)[37]	Orhan and Sahyyn (2001)[38] Browne et al (1999)[55]

REFERENCES

1. Gutteridge IBH. Antioxidants in nutrition, Health and Disease. Oxford, Oxford University Press, 1994.
2. Halliwell B. Free radicals and antioxidants: personal view. *Nutr Rev* 1994; 52: 253-256.
3. Bourdon E Blache D. The importance of proteins in defence against oxidation. *Antioxid Redox Signal* 2001; 3: 293-311.
4. Duchen MR. Role of mitochondria in health and disease. *Diabetes* 2004; 53 Suppl 1: 96-102.
5. Halliwell B, Gutterridge JMC. Free radical in biology and medicine. 3rd ed. New York: Oxford University Press., 1999.
6. Parker DV. Nutrition antioxidants and disease prevention: mechanism of action. In: Cansda TK (editor).Antioxidant in human health and disease 1999.
7. Duthie GG, Duthie SJ, Kyle JAM. Plant polyphenols in cancer and health disease: implications as nutritional antioxidant. *Nutr Res Rev* 2000; 13: 79-106.
8. Thomas JA. Estres oxidativo y defense oxidante. In: Nutricion en salud y enfermedad. 9th ed. McGraw-Hill, Mexico, 2002.
9. Simpson J, Narita S, Dean R. Long-lived reactive species on free-radical damaged protein. *Biochem J* 1992; 282: 621-624.
10. Gieseg S, Jeremy A, Dean R. Protein-bound 3, 4-dihydroxy-phenylalanine is a major reductant formed during hydroxyl radical damage to proteins. *Biochem* 1993; 32: 4780-6.
11. Davies M, Dean R. Radical-mediated protein oxidation from chemistry to medicine. Oxford: Oxford Univ. Press, 1997.
12. Kennedy TP, Rao NV, Noah W, Michael JR, Jafari M.J, Gurtner et al. *J Clin Invest* 1990; 86: 1565-1573.
13. Zapolska-Downar H, Naruszewicz D. A pleiotropic anti-atherogenic action of ibuprofen. *Med Sci Monit* 2001; 7(4): 837-841.
14. Dabhi JK, Solanki JK, Mehta A. Anti-atherosclerotic activity of ibuprofen, a non-selective COX inhibitor--an animal study. *Indian J. Exp Biol* 2008; 46(6): 476-481.
15. Dokmeci D, Kanter M, Inan M, Aydogdu N, Basaran UN, Yalcin O et al. Protective effects of ibuprofen on testicular torsion/detorsion-induced ischemia/reperfusion injury in rats. *Arch Toxicol* 2007; 81(9): 655-663.
16. Chang H, Yen JH, Yin CL. Celecoxib stimulates respiratory burst through pertussis toxin-sensitive G-protein, a possible signal for beta 2-integrin expression on human neutrophils. *Eur J Pharmacol* 2004; 484: 29- 39.
17. Needs CJ, Brooks PM. Clinical pharmacokinetics of the salicylates. *Clin Pharmacokinet* 1985; 10: 164-177.
18. Janice ED, Padidar S, Horgan G, Garry GD, Wendy RR, Reid M et al. Salicylate modulate oxidative stress in the rat colon: A proteomic approach. *Biochemi Pharmac* 2006; 2(2): 204-216.
19. Hsu CS, Li Y. Aspirin potently inhibit oxidative DNA strand breaks: implications for cancer chemoprevention. *Biochem Biophys Res Comm* 2002; 293: 705-09.
20. Hare LG, Woodside JV, Young IS. Dietary salicylates. *J Clin Pathol* 2003; 56: 649-650.
21. Peek Jr. RM. Prevention of colorectal cancer through the use of COX- 2 selective inhibitors. *Cancer Chemother Pharmacol* 2004; 54: 50-56.
22. Drew JE, Arthut JR, Farquharson AJ, Russell WR, Morrice PC, Duthie GG. Salicylic acid modulates oxidative stress and glutathione peroxidase activity in the rat colon. *Biochem Pharmacol* 2005; 70: 888-893.
23. Reilly ME, Patel VB, Peters TJ, Preedy VR. *In vivo* rates of skeletal muscle protein synthesis in rats are decreased by acute ethanol treatment but are not ameliorated by supplemental alpha-tocopherol. *J Nutr* 2000; 130: 3045-3049.
24. Zentella de Piña M, Hernández-Tobías A, Saldaña-Balmori Y, Díaz-Belmont A, Piña E. Biochemical ethanol effects affected by a non-steroidal anti-inflammatory drug, *FEBS Lett* 1992; 298: 123-125.
25. Zentella de Piña M, Saldaña-Balmori Y, Hernández-Tobías A, PiñaE. Nonsteroidal anti-inflammatory drugs lower ethanol-mediated liver increase in lipids and thiobarbituric acid reactive substances. *Alcohol Clin Exp Res,* 1993.
26. Zentella de Piña M, Corona S, Rocha-Hernández A, Saldaña-Balmori Y, Cabrera G, Piña E. Restoration by piroxicam of liver glutathione levels decreased by acute ethanol intoxication. *Life Sci* 1994; 54: 1433-1439.
27. Basu S. Theorized Mechanism of Non-Steroidal Anti-Inflammatory Drugs against Alzheimer's disease Onset and Progression. *J Young Invist* 2001; 3: 1-2.
28. Antwerpen P, Gelbcke M, Dubois J, Nève J. The reactions of oxicam and sulfoanilide non steroidal anti-inflammatory drugs with hypochlorous acid: determination of the rate constants with an assay based on the competition with para-aminobenzoic acid chlorination and identification of some oxidation products. *Free Radic Res* 2004; 38: 251-258.
29. Furst DE, Tino Muster DM. Non-steroidal anti-inflammatory drugs, diseases-modidiying antirheumatic drugs, non-opioids analgesics and drugs used in gout. In: Katzung BG (Eds). Basic and clinical pharmacology . Lange medical books/McGraw-Hill, 2001.
30. Van Der Vliet A, Bast A. Effects of oxidative stress on receptors and signal transmission. *Chem Biol Interactions* 1992; 85; 95-116.
31. Dana R, Malech HL and Levy R. The requirement for phospholipase A2 for activation of the assembled NADPH oxidase in human neutrophils. *Biochem J* 1994; 297; 217-223.
32. Chakraborty S, Roy K, Sengupta C. Exploring effects of different nonsteroidal antiinflammatory drugs on lipid peroxidation. Part II. 4-HNE profile. *Acta Pol Pharm* 2007; 64(3): 211-6.
33. Kadiiskaa MB, Gladena BC, Bairda DD, Grahama LB, Parkera CE, Amesb BN et al. Effects of the nonsteroidal anti-inflammatory agents indomethacin and meclofenamic acid on easurements of oxidative products of lipids in CCl4 poisoning. *Free Radic Biol Med* 2005; 38: 711-718.
34. Fernandes E, David Costa F, Sofia A Toste, José LFC Lima, Salette Reis. In vitro scavenging activity for reactive oxygen and nitrogen species by nonsteroidal anti-inflammatory indole, pyrrole, and oxazole

derivative drugs .*Free Rad Biol Med* 2004; 37: 1895-1905.
35. Parij N, Nagy AM, Neve J. Linear and non linear competition plots in the deoxyribose assay for determination of rate constants for reaction of nonsteroidal antiinflammatory drugs with hydroxyl radicals. *Free Radic Res* 1995; 23: 571.
36. Oktar BK, Cakir B, Mutlu N, Celikel C, Alican I. Protective role of cyclooxygenase (COX) inhibitors in burn-induced intestinal and liver damage. *Burns* 2002; 28(3): 209-14.
37. Kalapatapu VR, Satterfield L, Brown AT, Chen H , Ercal N, Price TO et al. The effects of Toradol on postoperative intimal hyperplasia in a rat carotid endarterectomy model. *Lab res Vasc Endovasc Surg* 2007; 41(5): 402-8.
38. Orhan H, Sahyyn G. In vitro effects of NSAIDS and paracetamol on oxidative stress-related parameters of human erythrocytes. *Exp Toxic Pathol* 2001; 53: 133-140.
39. Kamiyama T, Sato C, Liu J, Tajiri K, Miyakawa H, Marumo F. Role of lipid peroxidation in acetaminophen-induced hepatotoxicity: comparison with carbon tetrachloride. *Toxicol* 1993; 66: 7-12.
40. Wallace JL, Granger DN. Pathogenesis of NSAID gastropathy: are neutrophils the culprits. *Trends Pharmacol Sci* 1992; 13: 129-131.
41. Hickey EJ, Raje RR, Reid VE, Gross SM, Ray SD. Diclofenac- induced *in vivo* nephrotoxicity may involve oxidative stress-mediated massive genomic DNA fragmentation and apoptotic cell death. *Free Radic Biol Med* 2001; 31(26): 139-152.
42. Cantoni L, Valaperta R, Ponsoda X, Castell J.V, Barelli D, Rizzardini M et al. Induction of hepatic hemoxygenase-1 by diclofenac in rodents: role of oxidative stress and cytochrome P-450 activity. *Hepatology* 2003; 38: 776-783.
43. Wallace JL, McKnight W, Reuter BK, Vergnolle N. NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroent* 2000; 119: 706-714.
44. Somasundaram S, Hayllar H, Rafi S, Wrigglesworth JM, Macpherson AJ, Bjarnason I. The biochemical basis of nonsteroidal anti-inflammatory drug-induced damage to the gastrointestinal tract: a review and a hypothesis. *Scand J Gastroenterol* 1995; 30: 289-299.
45. Basivireddy J, Vasudevan A, Jacob M, Balasubramanian KA. Indomethacin-induced mitochondrial dysfunction and oxidative stress in villus enterocytes. *Biochem Pharmacol* 2002; 64: 339-349.
46. Jacob M, Bjarnason I, Simpson RJ. In vitro evidence for mitochondrial effects of indomethacin. *Biochem Soc Trans* 1998; 26: 315.
47. Jacob M, Bjarnason I, Simpson RJ. Effects of indomethacin on energy metabolism in rat and human jejunal tissue. *In vitro Clin Sci* 2001; 101: 493-498.
48. Miura S, Suematsu M, Tanaka S, Nagata H, Houzawa S, Sduzuki M, et al. Microcirculatory disturbance in indomethacin-induced intestinal ulcer. *Am J Physiol* 1991; 261: 213-219.
49. Nalini S, Mathan MM, Balasubramanian KA. Free radical-induced damage during intestinal ischaemia reperfusion in normal and xanthine oxidase deficient rats. *Mol Cell Biochem* 1993; 124: 59-66.
50. McCarthy DM. Mechanism of mucosal injury and healing: the role of non-steroidal anti-inflammatory drugs. *J Gastroenterol* 1995; 208: 24-29.
51. Fossline E. Adverse effects of non-steroidal anti inflammatory drugs on the gastrointestinal system. *Ann Clin Lab Sci* 1998; 28: 67-81.
52. Hermann M, Kapiotis S, Hofbauer R, Seelos C, Held I, Gmeiner B. Salicylate promote myeloperoxidase-initiated LDL oxidation: antagonization by its metabolite gentisic acid. *Free Radic Biol Med* 1999; 26: 1253-1260.
53. Nair P, Singh KS, Nath SS. Effects of non steroidal anti-inflammatory drugs on the antioxidant defence system and the membrane functions in the rat intestine. *Nutr Hosp* 2006; 21(6): 638-649.
54. Jiménez MD, Martín MJ, Alarcón de la Lastra C, Bruseghini L, Esteras A, Herrerías JM, et al. Role of L-arginine in ibuprofen-induced oxidative stress and neutrophil infiltration in gastric mucosa. *Free Radic Res* 2004; 38(9): 903-11.
55. Browne GS, Nelson C, Nguyen T, Ellis BA, Day RO, Williams KM. Stereoselective and substrate-dependent inhibition of hepatic mitochondria beta-oxidation and oxidative phosphorylation by the non-steroidal anti-inflammatory drugs ibuprofen, flurbiprofen, and ketorolac. *Biochem Pharmacol* 1999; 57(7): 837-44.
56. Bandyopadhyay D, Ghosh G, Bandyopadhyay A, Reiter RJ. Melatonin protects against piroxicam-induced gastric ulceration. *J Pineal Res* 2004; 36(3): 195-203.
57. Mingatto FE, Santos AC, Uyemura SA, Jordani MC, Curti C.In vitro interaction of nonsteroidal anti-inflammatory drugs on oxidative phosphorylation of rat kidney mitochondria: respiration and ATP synthesis. *Arch Biochem Biophys* 1996; 334: 303-308.
58. Hickey EJ, Raje RR, Reid VE, Gross SM, Ray SD. Diclofenac- induced *in vivo* nephrotoxicity may involve oxidative stress-mediated massive genomic DNA fragmentation and apoptotic cell death. *Free Radic Biol Med* 2001; 31(26): 139-152.
59. Ishiyama H, Ogino K, Shimomura Y, Kanke T, Hobara T. Hepatotoxicity of diethyldithiocarbamate in rats. *Pharmacol Toxicol* 1990; 67: 426-430.
60. Masubuchi Y, Horie T. A possible mechanism of naproxen-induced lipid peroxidation in rat liver microsomes. *Pharmacol Toxicol* 2001; 89: 43-48.
61. Cantoni L, Valaperta R, Ponsoda X, Castell JV, Barelli D, Rizzardini M, et al. Induction of hepatic hemoxygenase-1 by diclofenac in rodents: role of oxidative stress and cytochrome P-450 activity. *Hepatology* 2003; 38: 776-783.
62. Gomez-Lechon MJ, Ponsoda XOCE, Donato T, Castell JV, Jover R. Diclofenac induce apoptosis in hepatocytes by alteration of mitochondrial function and generation of ROS. *Biochem Pharmacol* 2003; 66: 2155-2167.
63. Bort R, Ponsoda X, Jover R. Diclofenac toxicity to hepatocytes: a role for drug metabolism in cell toxicity. *J Pharmacol Exp Ther* 1999; 288: 65-72.
64. Masubuchi Y, Yamada S, Horie T. Possible mechanisms of hepatocyte injury induced by diphenylamine and its structurally related NSAIDS. *J Pharmacol Exp Ther* 2000; 292: 982-7.

65. Thomas SHL. Paracetamol (acetaminophen) poisoning. *Pharmac Ther* 1993; 60: 91-120.
66. Nelson SD. Molecular mechanisms of the hepatotoxicity caused by acetaminophen. *Semin Liver Dis* 1990; 10: 267-278.
67. Jollow DJ, Mitchell JR, Potter WZ, Davis DC, Gillette JR, Brodie BB.Acetaminophen-induced hepatic necrosis. II. Role of covalent binding *in vivo*. *J Pharmacol Exp Ther* 1973; 187: 195-202.
68. Ajith TA, Hema U, Aswathy MS. Zingiber officinale Roscoe prevents acetaminophen-induced acute hepatotoxicity by enhancing hepatic antioxidant status. *Food Chem Toxicol* 2007; 45(11): 2267-72.
69. Yokoyama H, Horie T, Awazu S. Lipid peroxidation in rat liver microsomes during naproxen metabolism. *Biochem Pharmacol* 1993; 45: 1721-1724.
70. Yokoyama H, Horie T, Awazu S. Lipid peroxidation and chemiluminescence during naproxen metabolism in rat liver microsomes. *Hum Exp Toxicol* 1994; 13: 831-838.
71. Yokoyama H, Horie T, Awazu S. Oxidative stress in isolated rat hepatocytes during naproxen metabolism. *Biochem Pharmacol* 1995; 49: 991-996.
72. Ji B, Masubuchi Y, Horie T. A possible mechanism of naproxen-induced lipid peroxidation in rat liver microsomes. Pharmacol Toxicol 2001; 89(1): 43-8.
73. Yokoyama H, Horie T, Awazu S. Glutathione disulfide formation during naproxen metabolism in the isolated rat hepatocytes. *Res Commun Mol Pathol Pharmacol* 1998; 99: 143-154.
74. Muller-Peddinghaus R, Wurl M. The amplified chemilumi-nescence test to characterize antirheumatic drugs as oxygen radical scavengers. *Biochem Pharmacol* 1987; 36: 1125-1132.
75. Nair P, Singh KS and Nath SS. Effects of non steroidal anti-inflammatory drugs on the antioxidant defense system and the membrane functions in the rat intestine. *Nutr Hosp* 2006; 21(6): 638-649.
76. Wasil M, Halliwell B and Moorhaus CP. Biologically-significant scavenging of the myeloperoxidase-derived oxidant hypochlorous acid by some anti-inflammatory drugs. *Biochem Pharmacol* 1987; 36: 3847-3850.
77. Balasubramanian T, Somasundaram M, Felix AJ. Taurine prevents Ibuprofen-induced gastric mucosal lesions and influences endogenous antioxidant status of stomach in rats. *Sci World J* 2004; 4: 1046-54.
78. Jiménez MD, Martín MJ, Alarcón De La Lastra C, Bruseghini L, Esteras A, Herrerías JM,; Motilva V. Role of l -Arginine in Ibuprofen-induced Oxidative Stress and Neutrophil Infiltration in Gastric Mucos. Free Radical Research, 2004; 38(9): 903-911.
79. Ishiyama H, Ogino K, Shimomura Y, Kanke T and Hobara T. Hepatotoxicity of diethyldithiocarbamate in rats. *Pharmacol Toxicol* 1990; 67: 426-430.