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STUDIES ON THE EFFECT OF CIPROFLOXACIN ON THE BIOCHEMICAL PARAMETERS IN TESTIS OF ALBINO RATS

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Abstract

Healthy adult male albino rats of wistar stain weighing 180 – 225gms were used. They were fed with a standard balanced diet and clean drinking water. The drug ciprofloxacin hydrochloride T.P was used for the animals. The weighed animals divided into the four groups, of five animals each and received of treatment. After treatment of weighed the rats, 24 hrs after the last treatment schedule the animals were sacrificed by decapitation. The removed testis washed in 0.9% saline, freed from the adhering connective tissue mass and blotted on a filter paper. The organ was weighed and stored at 4°C until used for further biochemical analysis such as Testicular Lipid peroxidation (LP), Superoxide dismutase (SOD), Catalase (CAT) and Glutathione (GSH). In the present study ciprofloxacin treatment brought about an increase in lipid peroxidation in a dose and duration, both groups are (short and long duration) LP was increasing, but the enzymatic and non enzymatic markers like Superoxide dismutase, Catalase and Glutathione also was significantly reduced by ciprofloxacin at both treatments when compared to control. SOD, CAT and GSH all the vitamin supplementations were capable of increasing the activity to near control levels.

Key Words: Ciprofloxacin, Lipid peroxidation, Superoxide dismutase, Catalase, Glutathione.

INTRODUCTION

NSAIDs are one such group of drugs that are widely prescribed, with some available without a prescription (Hubbell, 1996). These drugs are used to suppress the signs and symptoms of inflammation and the accompanying pain and fever (Painham, 1991).

Infertility may sometimes be associated with NSAID consumption during their child bearing years (Akil *et al.,* 1996). As inhibitors of cyclooxygenase NSAIDs given during pregnancy have the potential to cause adverse maternal and foetal effects. Maternal effects include prolongation of pregnancy and labour (Needs and Brooks, 1985) where a constriction of the ductus arteriosus, renal dysfunction and haemostatic abnormalities can occur in the foetus and neonate (Ostensen and Ostensen, 1996). NSAIDs are excreted in small amounts into breast milk with little risk for adverse effects in the sucking infant (Ostensen, 1998).

Currently, no NSAID is available that lacks potential for serious toxicity; therefore, long term use of NSAIDs should be avoided whenever possible, particularly in high-risk patients. e.g., those who are elderly, suffer from hypertension, congestive heart failure, renal or hepatic impairment or volume depletion, take certain concomitant medications or have a history of peptic ulcer disease (Tannenbaum *et al.,* 1996).

Recent epidemiological studies suggest that non-steroidal anti-inflammatory drugs (NSAIDs) reduce the risk of several cancers including breast cancer (Khuder and Mutgi, 2001).

Ciprofloxacin is highly active against *Enterobacteriaceae*, including *salmonella* and *shigellae* and *Haemophilus influenzae*, irrespective of ampicillin resistance (Beskid *et al.,* 1993). More than 85% of *Enterobacteriacaeae* are susceptible, although there are local variations (Prosser *et al.,* 1995). Ciprofloxacin is also the most active 84% of the quinolones against *Pseudomonas aeruginosa* (Beskid *et al.,* 1993).

Ciprofloxacin is active against most *staphylococci* and *streptococci*, but is less effective against the *Enterococci* (Prosser *et al.,* 1995). Oxacillin sensitive *Staphylococcus aureus* is usually susceptible by 96% but most oxacillin-resistant strains are also resistant to ciprofloxacin (Beskid and Prosser, 1993).

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Ciprofloxacin is much less active against *Streptococcus pneumonia* than other respiratory pathogens (58%) and concentrations that can be attained in the respiratory tract are only one-third of the mean inhibitory concentration (MIC) (Cherubin *et al.,* 1992). *Listeria spp* and *Mycobacterium* spp. are not susceptible to ciprofloxacin than other quinolones (Klopman *et al.,* 1994).

In an animal study, administration of an extract of the whole plant dandelion (actually *Taraxacum, mongolicum*), a close relative of the more common western dandelion, (*Taraxacum officinale*) concomitantly with ciprofloxacin decreased absorption of the drug. Preliminary research in animals has shown that fennel may also reduce the absorption of ciprofloxacin. The authors found this was due to the high mineral content of the herbs (Zhu *et al*., 1999).

MATERIALS AND METHODS

Healthy adult male albino rats of Wistar strain weighing 180 - 225 gms were used in the present investigation. They were housed in clean cages in a well ventilated room with 12 ± 1 hour light and 12 ± 1 hour dark schedule. They were fed with a standard balanced diet and clean drinking water was made available *ad libitum*. The drug Ciprofloxacin hydrochloride T.P. manufactured by Okasa Limited, Goa, India was used for the The animals were weighed and divided into the following four groups, of 5 animals each, and received the following regimen of treatment.

Group I: Control: Rats received distilled water and oil orally, respectively.

Group II: Short Duration: Rats received ciprofloxacin hydrochloride for one week

Group III: Short Duration + Withdrawal: Treatment as for group IV plus 14 days of drug withdrawal

Group IV: Long Duration: Rats received ciproflaxacin hydrochloride for four weeks

Group V: Long Duration +Withdrawal: Treatment as for group IV plus 60 days of drug withdrawal.

Group II and Group IV were further subdivided into five sub groups.

1. Low dose ciprofloxacin treated group : The rats received ciprofloxacin (250 mg/60 kg body weight)
2. High dose ciprofloxacin treated group : The rats received ciprofloxacin (400 mg/60 kg body weight)
3. High dose ciprofloxacin + vitamin 'A' treated group: The rats received ciprofloxacin (400 mg / 60 kg body weight) and vitamin 'A' (7.5 mg / 60 kg body weight), respectively.
4. High dose ciprofloxacin + vitamin 'C' treated group: The rats received ciprofloxacin (400 mg/60 kg body weight) and vitamin 'C' (500 mg / 60 kg body weight), respectively.
5. High dose ciprofloxacin + vitamin 'E' treated group: The rats received ciprofloxacin (400 mg/60 kg body weight) and vitamin 'E' (600 mg / 60 kg body weight), respectively.

Short duration treatment rats received ciprofloxacin orally for seven consecutive days and long duration treatment rats received the same dosage of ciprofloxacin orally for 4 weeks. And further the drug was withdrawn for the next 14 days and 60 days, respectively, Experiment. The drug was dissolved in distilled water and was administered orally.

CHEMICALS AND REAGENTS

All chemicals and reagents used for the experiments were of analytical grade and were obtained from BDH (British Drug House, England and India), E. Merck (Germany and India), Sigma chemical company (USA), Loba chemie (Indo austranol Co, India) Qualigens fine chemicals division (Mumbai).

EXPERIMENTAL PROCEDURE

The animals were weighed before and after treatment. Twenty four hours after the last treatment schedule the animals were sacrificed by decapitation method. The removed testis washed in 0.9% saline, freed from the adhering connective tissue mass and blotted on a filter paper. The organ was weighed and stored at 4°C until used for further biochemical analysis

EXTRACTION OF THE ENZYME FROM TISSUE

The testis tissue were homogenised in 0.25 M sucrose using Potter Elvejhem homogenizer. The homogenates were frozen and thawed thrice and centrifuged at 1000 x g for 10 min at 4°C. The supernatant was filtered and the filterates were kept frozen at 5°C before analysis.

BIOCHEMICAL ANALYSIS

The levels of lipid peroxidation in testis are estimated

by the method of Nichens and Samuelson (1968). The activity of superoxide dismutase (SOD; EC 1.15.1.1) was assayed by the method of Kakkar *et al.* (1984). Catalase (CAT; EC.1.11.1.6.) was assayed colorimetrically by the method of Sinha (1972) and Total glutathione (reduced) in testis was determined according to the method of Beutler and Kelley (1963).

RESULTS

Effect on Testicular Lipid peroxidation (LP) (Table.1)

Ciprofloxacin treatment brought about an increase in lipid peroxidation (LP) in a dose and duration dependent manner. In the short duration group, a 2 to 3 fold increase in LP was evident, however, a 5 to 9 fold elevation of the same was observed in the long duration drug treated groups. In short duration groups neither drug withdrawal nor vitamin supplementations had any restorative effect. However, in long duration groups all the vitamins (A, C, E) were very efficient in bringing back the lipid peroxidation to near normalcy. The withdrawal of the drug was ineffective in changing the drug effects.

Long duration treatments

Unlike short duration changes induced by Ciprofloxacin, a significant lowering of the SOD activity was observed in a dose dependent manner when the drug was given for a longer period. Neither drug withdrawal nor vitamin supplementations could stimulate the enzymatic activity back to control levels.

Effect on Testicular Catalase (CAT) Activity (Table.1)

Short duration treatment

Ciprofloxacin treatment caused a decrease in testicular catalase activity in both low (23%) and high dose (48%), groups of animals, respectively. Only vitamin C could restore the enzymatic activity back to control levels. Whereas, drug withdrawal as well as supplementation with vitamins A and E could raise the enzymatic activity only partially to the level observed in low dose drug treated rats.

Long duration treatment

The drug administration for longer duration had drastic effect on catalase activity, since, 74 to 80% decrease was observed at both the doses. Neither drug withdrawal nor vitamin supplementations had any marked restorative effect.

Table1 Effect of Ciprofloxacin with and without vitamins supplementation on Testicular Lipid Peroxidation, Superoxide Dismutase, Catalase activity and Glutathione concentration in rats

GROUPS	TREATMENTS	LIPIDPEROXIDATION (n moles / mg. tissue)	SUPEROXDE DISMUTASE (n moles / min / mg. protein)	CATALASE (n moles / min / mg. protein)	GLUTATHIONE (n moles / mg. tissue)
I	***Control***	$0.276^{i} \pm 0.005$	$1.552^{a} \pm 0.018$	$0.136^{a} \pm 0.004$	$0.0480^{a} \pm 0.0004$
II	***Short duration*** Low dose	$0.723^{g} \pm 0.001$	$1.414^{d} \pm 0.0008$	$0.105^{c} \pm 0.001$	$0.0323^{e} \pm 0.0005$
	High dose	$1.153^{e} \pm 0.001$	$1.312^{e} \pm 0.003$	$0.092^{e} \pm 0.0002$	$0.0342^{d} \pm 0.0007$
	High dose + vitamin 'A'	$0.981^{f} \pm 0.001$	$1.473^{c} \pm 0.002$	$0.99^{d} \pm 0.0003$	$0.0413^{b} \pm 7.0700$
	High dose + vitamin 'C'	$0.665^{h} \pm 0.002$	$1.496^{b} \pm 0.004$	$0.129^{b} \pm 0.003$	$0.0383^{c} \pm 0.0004$
	High dose + vitamin 'E'	$0.780^{g} \pm 0.002$	$1.423^{d} \pm 0.003$	$0.087^{f} \pm 0.001$	$0.0263^{g} \pm 0.0008$
III	Withdrawal	$1.688^{c} \pm 0.008$	$1.420^{d} \pm 0.0008$	$0.106^{c} \pm 0.0007$	$0.0294^{f} \pm 0.0002$
IV	**Long duration** Low dose	$1.556^{d} \pm 0.002$	$0.307^{i} \pm 0.001$	$0.035^{h} \pm 0.0003$	$0.0130^{i} \pm 0.0003$
	High dose	$2.448^{b} \pm 0.001$	$0.287^{i} \pm 0.002$	$0.026^{i} \pm 0.0006$	$0.0090^{i} \pm 0.0002$
	High dose + vitamin 'A'	$0.248^{i} \pm 0.002$	$0.328^{h} \pm 0.002$	$0.038^{h} \pm 0.0001$	$0.0176^{h} \pm 0.0004$
	High dose + vitamin 'C'	$0.227^{i} \pm 0.002$	$0.402^{f} \pm 0.003$	$0.045^{g} \pm 0.0003$	$0.0081^{i} \pm 0.0001$
	High dose + vitamin 'E'	$0.239^{i} \pm 0.001$	$0.318^{hi} \pm 0.003$	$0.033^{h} \pm 0.0002$	$0.0086^{i} \pm 0.0005$
V	Withdrawal	$2.937^{a} \pm 0.001$	$0.347^{g} \pm 0.001$	$0.037^{h} \pm 0.0001$	$0.0138^{i} \pm 0.0001$

Each value in the mean ±SE; Means followed by a common letter are not significantly different at the 5% Level of DMRT.

Effect on Testicular Superoxide dismutase (SOD) Activity (Table.1)

Short duration Treatment

Ciprofloxacin administration caused a lowering of the SOD activity by 15% only in the high dose group. This decrease was compensated slightly by drug withdrawal.

All the vitamin supplementations were capable of increasing the SOD activity to near control levels.

Effect on Testicular Glutathione (GSH) (Table.1)

Like the enzymatic systems (SOD and Catalase), the testicular glutathione also was adversely affected by ciprofloxacin at both short and long duration treatments, in a dose and duration dependent manner. The glutathione levels were significantly reduced i.e., 30 to 33% reduction in short duration

group and 73 to 81% reduction in long duration group. Only vitamin A could partially raise the glutathione concentration in the high dose drug treated groups. Drug withdrawal was ineffective in bringing any change in the glutathione concentration induced by the drug.

DISCUSSION

Effect on Testicular Lipid peroxidation

Lipid peroxidation occurs mainly in membranes, where the content of unsaturated fattyacids is relatively high. Peroxidation of membrane lipids arising out of oxidative damage in intact cells results in decreased fluidity, inactivation of membrane bound enzymes and receptors and changes in nonspecific ion permeability. Greater the unsaturation, greater is the lipid peroxidation. Cumulative effects of lipid peroxidation have been implicated as underlying mechanisms in various pathological conditions like atherosclerosis, haemolytic anemias and ischemia (Halliwell, 1993). Cellular antioxidant defense systems such as superoxide dismutase, and antioxidants such as tocopherol and β carotene are considered to play a significant role in quenching reactive oxygen species (ROS) thereby displaying a modulatory role in many of the disease conditions (Shanidi *et al*., 1992).

When the endogenous respiration of washed suspensions of bovine or ovine spermatozoa is followed manometrically over several hours at 37°C, a gradual decline in the rate of oxygen uptake and in motility is invariably registered; this can be prevented if the sperm suspension is supplemented with an oxidizable substrate such as fructose, glucose, lactate, pyruvate or acetate, all of which are capable of sustaining exogenous sperm respiration (Mann and Lutwak - Mann, 1948).

It was also noted by Masaki (1974) and others that during prolonged storage of bovine or ovine spermatozoa at 4°C (the temperature at which semen is stored prior to artificial insemination), their content of phospholipid, especially plasmalogen, decreases move quickly in samples losing their motility faster. The falling rate of endogenous respiration and poor storability may be a result of the afore-mentioned decrease in acylester bonds of sperm phospholipid, when lysoplasmalogen is liberated, a metabolite which is notoriously harmful to spermatozoa (Hartree and Mann, 1960). Another plausible explanation is that during aerobic incubation the sperm phospholipid undergoes peroxidation, which leads to the formation of toxic fatty acid peroxides. This is borne out by observations on both animal and human spermatozoa, indicating that mammalian spermatozoa are highly susceptible to lipid peroxidation and that peroxidized fatty acids are strongly spermicidal (Mann *et al*., 1980).

Coincidentally with lipid peroside formation, motility and metabolism in the sperm suspension decline and certain intracellular enzymes escape from the spermatozoa through the damaged plasma membrane. The peroxidation is accompanied by a fall in phospholipids roughly two-thirds of their lipid-P and half their content of plasmalogen, palmitaldehyde and major unsaturated fatty acids such as decosahexaenoic and arachidonic acid (Jones and Mann, 1976).

Similarly, a detrimental effect on motility develops when a fresh suspension of motile ram spermatozoa is treated with peroxidized decosahexaenoic arachidonic or other unsaturated fatty acids; toxicity caused in this way is directly related to the degree of fatty acid peroxidation, and manifests itself by an instantaneous arrest of motility and loss of respiratory and fructolytic activity (Jones and Mann, 1977).
In the present study, there was a remarkable increase in testicular lipid peroxidation process and a lowered glutathione concentration after ciprofloxacin treatment at low and high doses and this effect was persistent even after drug withdrawal. The lowered glutathione suggests an inhibition of glutathione induced cell protection against oxidative damage and membrane lipid peroxidation. Lipid peroxidation observed in this study indicates increase in the loss of essential fatty acids, erosion of antioxidant protection and increase in the membrane leakiness etc. (Mead, 1976). The increased lipid peroxidation also indicates a depletion of vitamins especially vitamin E due to the drug effect, as supplementation with vitamin E could lower the TBA materials to control levels.

Mammalian cells accumulate ascorbate from tissue fluids against a concentration gradient coupled to uptake of Na^+. Gut absorption of ascorbate is also Na^+- dependent. Several cell types (especially neutrophils) also rapidly take up oxidized ascorbate (DHA) if it is present and may use some of the glucose transport systems to do so (Rumsey *et al*., 1997). The most striking chemical property of ascorbate is its ability to act as a reducing agent. The observation that dietary ascorbate inhibits the carcinogenic action of several nitroso-compounds fed to animals can in part be attributed to its ability to reduce them to inactive forms. *In vitro*, ascorbate has been shown to

have a multiplicity of antioxidant properties, protecting various biomolecules against damage by both ROS and RNS. The levels of ascorbate found *in vitro* (30-100 µM in human plasma; higher in CSF, aqueous humour of the eye, gastric juice and lung lining fluid; millimolar levels intracellularly in many cell types) are sufficient to exert such antioxidant effects, i.e. an antioxidant action of ascorbate is feasible *in vivo*. However, direct evidence that ascorbate does act as an antioxidant *in vivo* is limited.

Nevertheless, feeding to guinea-pigs or ODS rats on a diet restricted in vitamin C has been reported to decrease the vitamin E content of tissues, consistent with an interaction between the two vitamins *in vivo* (Tanaka *et al*., 1997). An early study on guinea-pigs showed that a vitamin C-deficient diet led to increased exhalation of pentane and ethane, suggestive of increased lipid peroxidation *in vivo*. Further evidence for an antioxidant role of ascorbate *in vivo* is provided by studies of its depletion under conditions of oxidative stress (Schorah *et al*., 1996). Thus ascorbate becomes oxidized to DHA in synovial fluid in the knee-joints of patients with active rheumatoid arthritis and in the lungs of patients with adult respiratory distress syndrome. Presumably ascorbate is acting to scavenge ROS / RNS derived from the many activated phagocytes present. Measurement of ascorbyl radical by ESR has been used as an index of oxidative stress in several systems (Halliwell and Gutteridge, 1999).

Vitamin E, as a scavenger of peroxyl radicals, is probably the most important (but not the only), inhibitor of the free-radical chain reaction of lipid peroxidation in animals. Initiation of lipid peroxidation can be prevented by enzymes that savenger ROS / RNS and proteins that sequester transition metal ions. Sequestration of metal ions also prevents them from decomposing perioxides into chain-propagating peroxyl and alkoxyl radicals. The dietary content of vitamin E is one factor that affects the sensitivity of laboratory animals to certain toxins or to tissue insults such as ischaemia reperfusion (Halliwell and Gutterdge, 1999).

In animals, many of the signs of vitamin E deficiency can be partly or completely alleviated by feeding synthetic chain-breaking antioxidants (e.g. ethoxyquin or promethazine) or by raising the selenium content of the diet. It is well-known to veterinary practitioners, zoo-keepers and farmers that feeding unsaturated fats to animals increases their requirement for vitamin E. Feeding more vitamin E to pigs, chickens and cows has been reported to increase the stability of their meat against rancidity on storage (Schaefer *et al*., 1995). Tissue samples taken from vitamin E-deficient animals show evidence of peroxidation (e.g. as elevated levels of isoprotanes or, less convincingly as TBA-reactive material) and tissue homogenates or subcellular fractions from such animals peroxidize more rapidly than normal when incubated *in vitro*. Vitamin E - deficient animals are more sensitive to the toxic effects of pure O_2. Vitamin E deficient rats exhale more hydrocarbon gases and accumulate fluorescent pigments in certain tissues more rapidly than normal, especially if they are fed a diet rich in PUFA's. Lack of vitamin E in the diets of rodents increases the rate of accumulation of Senescent Cell antigen, a cell-surface protein indicative of 'old' cells. It is a breakdown product of the membrane ion transport protein band 3 (Halliwell and Gutteridge, 1999).

So vitamin C appears to be an important antioxidant system. In the present study, vitamin C, E and A supplementations were effective in reducing the ciprofloxacin induced lipid peroxidation in the testis by expressing their antioxidant property effectively, especially when given for longer durations.

Many drugs are known to adversely affect the testicular cells by lipid peroxidation. Milton Prabu (1997) has shown cadmium chloride administration to cause tissue damage through lipid peroxidation Cheol park *et al*. (2003) have reported that rebamipide is an effective free radical scavenger and is known to inhibit the endogenous ROS production and the lipid peroxidation of cell membrane is sperms and to enhance the sperm motility during sperm processing and cryopreservation. Vitamin E administration causes significant improvement in sperm motility and viability according to Verma and Kanwar (1999). Verma and Nair (2002) have seen a similar adverse effect of aflatoxin induced lipid peroxidation in the testis of mice. Many workers have observed an increase in the hydrogen peroxide (H_2O_2) generation in the testis after administration of insecticides like lindane (Sujatha *et al*., 2001) endosulfan and methoxychlor (Chitra *et al*., 1999).

Effect on Testicular Superoxide dismutase (SOD)

SOD is an important antioxidant enzyme since, SOD appears specific for $O^{\bullet}{}_2^-$ as a substrate, it follows that $O^{\bullet}{}_2^-$ is a species that is deleterious *in vivo*. When compared with such highly reactive species as $OH^{\bullet}$,

$O^{\bullet}_{2}$ seems innocuous in chemical terms.

The protonated from of $O^{\bullet}_{2}$, $OH^{\bullet}$, is somewhat more reactive than $O^{\bullet}_{2}$ itself.

For example, $HO^{\bullet}_{2}$ can initiate peroxidation of fatty acids and a small amount of $OH^{\bullet}_{2}$ exists in equilibrium with $O^{\bullet}_{2}$ even at physiological pH. The pH close to a membrane surface may be more acidic than the pH in bulk solution. So that $OH^{\bullet}_{2}$ formation would be favoured. The pH beneath activated macrophages adhering to a surface has been reported to be ≤5, and so a considerable amount of any $O^{\bullet}_{2}$ that they generate will exist as $HO^{\bullet}_{2}$. $HO^{\bullet}_{2}$ should be able to cross membranes as easily as H_2O_2. Much of the $O^{\bullet}_{2}$ generated within cells comes from membrane – bound systems (e.g. the electron – transport chains of mitochondria and endoplasmic reticulum), and so $HO^{\bullet}_{2}$ formed close to the membrane could conceivably produce damage. Any $O^{\bullet}_{2}$ that was produced in the hydrophobic membrane interior could be very damaging, since $O^{\bullet}_{2}$ is highly reactive in organic solvents. However, it has not been demonstrated that $O^{\bullet}_{2}$ or $HO^{\bullet}_{2}$ mediate direct membrane damage *in vivo* (Flint *et al*., 1993).

Superoxide can decrease the activity of other antioxidant defence enzymes, such as catalase and glutathione dehydratase, aconitase, fumarase and dihydroxyacid dehydratase (Flint *et al*., 1993). Dihydroxyacid dehydratase, which catalyses the third step in the biosynthesis of branched chain amino acids, contains a $(4Fe\text{-}4s)^2$ + iron sulphur cluster at its active site, which is degraded upon exposure to $O^{\bullet}_{2}$ or (with a lower rate constant) to O_2 itself. Thus, energy metabolism in the krebs cycle is a major target of damage by $O^{\bullet}_{2}$. Inactivation is caused by oxidation of the cluster, leading to release of iron ions. Mammalian aconitase (Gardner *et al*., 1994) has a similar cluster and inactivates NADH dehydrogenase in bovine heart submitochondrial particles (Zhang *et al*., 1990). Hence energy metabolism may be a target of direct damage by $O^{\bullet}_{2}$ in mammalian cells also.

Another important target of damage may be ribonucleotide reductase.

This enzyme, needed to provide the precursors required for DNA synthesis, has an essential tyrosine radical at its active site. The reductase is inactivated by exposure to free radicals that can combine with the tyrosine radical: $NO^{\bullet}$ is one example and $O^{\bullet}_{2}$ may be another. Both *E. coli* and mammalian ribonucleotide reductases have been shown to be inactivated by $O^{\bullet}_{2}$ generating systems *in vitro*. Some mammalian creative kinases are also reported as inactivated by exposure to $O^{\bullet}_{2}$. Superoxide may also damage Calcineurin, a protein involved in signal transduction (Gaudu *et al*., 1996).

In the present study, ciprofloxacin had brought about an decrease in SOD activity in testis. The reduced SOD enzyme levels suggests poor conversion of superoxide anions to hydrozen peroxide. The reduced catalase activity indicates a lowered metabolism of hydrogen peroxide produced by SOD action to water and molecular oxygen. The reduced availability of glutathione as cofactor for the catalase induced metabolism further confirms the inactivation of the anti-oxidant mechanisms by the drug ciprofloxacin and production of more and more of reactive oxygen species and oxidative stress in the tissue.

Ciprofloxacin induced decrease in SOD activity was compensated by both drug withdrawal. Co-administration with vitamin C, E and A had protective effect against the drug induced ROS generation and oxidative stress. The pivotal roles of nutritional antioxidants are clearly understood, as evidenced by the antioxidative function of vitamin E (Bhardwaj *et al*., 2000; Verma and Nair, 2002), which is frequently used as a food additive as well as supplement to protect against oxidation. Vitamin A and C also have antioxidative functions (Das *et al*., 2002).

These compounds react directly with ROS and decrease their toxicity. Vitamin supplementations in the present study have also reacted directly with the ROS and hence the increasing SOD activity observed.

Many drugs are known to adversely affect the testicular spermatozoa, production of spermatogenesis and spermiogenesis. Chitra *et al*. (2003) have shown bisphenol

A administration to cause an increase in oxidative stress in epididymis resulting in degeneration of epididymal epithelium in rats. Co-administration with vitamin C had a protective effect against the drug induced toxicity in epididymal sperm and tissue.

The epididymis and spermatozoa are highly rich in polyunsaturated fatty acid and thus susceptible to damages induced by ROS. To counteract the effects of ROS the epididymis and spermatozoa are

equipped with antioxidant defence systems, namely superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. Increased lipid peroxidation may indicate an increased generation of free oxygen radicals in the epididymis, which has been associated with sperm mid-piece abnormalities and count decline (Thiele *et al*., 1995). Sujatha *et al*. (2001) in their studies indicate that lindane alters testicular functions possibly by inducing reactive oxygen species and decreasing the antioxidant enzymes thereby disrupting male reproduction.

Effect on Testicular Catalase (CAT)

Animal catalases consist of four protein subunits, each of which contains a ferric haem group bound to its active site (Reid *et al.,* 1981). The haem groups are buried in non-polar pockets, connected to the surface by narrow channels, thus preventing most molecules larger than H_2O_2 from gaining access . Each subunit usually has one molecule of NADPH bound to it. Dissociation of catalase into its subunits, which easily occurs on storage, freeze – drying or exposure of the enzyme to acid or alkali, causes loss of catalase activity (Sichak and Dounce, 1987).
The catalase activity of animal and plant tissues is largely or completely located in subcellular organelles bounded by a single membrane and known as peroxisomes (Chan *et al.,* 1979). Peroxisomes contain many of cellular enzymes that generate H_2O_2, such as glycolate oxidase, urate oxidase (not in primates) and the flavoprotein dehydrogenases involved in the β-oxidation of fatty acids, a metabolic pathway that operates in both mitochondria and peroxisomes in animal tissues. In mitochondria the flavoproteins involved in β-oxidation donate electrons to the electron – transport chain, but in peroxisomes they react with O_2 to give H_2O_2. It seems logical that these enzymes have been packaged into an organelle with high capacity to destroy H_2 O_2. Some Cu Zn SOD may also be present in peroxisomes.

In the present study, ciprofloxacin had brought about an decrease in CAT activity in testis. The reduced CAT activity exclusively detoxifies hydrogen peroxide and has no electron donor requirement. Although CAT is a well known antoxidative enzyme and has been implicated in protection against hydrogen peroxide, its localization is limited to the peroxisome. The reduced SOD levels a suggests poor conversion of superoxide anions produced to hydrogen peroxide. Further, reduced catalase activity indicates lowered metabolism of hydrogen peroxide produced by SOD action to water and molecular oxygen.

Ciprofloxacin induced decrease in CAT activity was compensated by drug withdrawl. Co-administration of vitamin C, E and A had protective effect against the drug induced ROS generation and oxidative stress. This work if corroborated with reports from many workers vitamin E protects against the ROS mediated damage on spermatozoa (Verma and Kanwar, 1999). Co-administration with vitamin C had a protective effect against the bisphenol A induced toxicity in epididymal sperm and epididymis as per Chitra *et al.* (2003). The protective effect of vitamin E on lipid peroxidation in the testis of aflatoxin treated mice has been reported by Verma and Nair (2002).

Many drugs are known to adversely affect the testicular spermatozoa, production of spermatogenesis and spermiogenesis. Sujatha *et al.* (2001) have shown lindane administration to cause an increase in oxidative stress and decreases in, antioxidant enzymes in adult male rats. Catalase activity has also been determined in human spermatozoa and seminal plasma (Jeulin *et al.,* 1989) of normal and infertile males. In terms of their site of synthesis, SOD and catalase (Zini *et al.,* 2002) are equally present in seminal plasma from fertile and vasectomized males thus suggesting a post – testicular secretion of these enzymes. According to Saleh and Agarwal (2002), the generation of reactive oxygen species (ROS) in the male reproductive tract has become a real concern because of their potential toxic effects at high levels on sperm quality and function. Fujii *et al*. (2003) have shown detoxifying Carbonyl compounds produced by oxidative stress, are present at high levels in the epithelia of the general tract and sertoli cells of the testis. Milton Prabu (1997) has shown cadmium chloride to induce oxidative stress and decrease in antioxidant enzyme like catalase in adult male rats.

Effect on Testicular glutathione (GSH)

Apart from its role as a cofactor for the glutathione peroxidase family, GSH is involved in many other metabolic processes, including ascorbic acid metabolism, maintaining communication between cells (Barhoumi *et al*. 1993), and in generally preventing protein – SH groups from oxidizing and cross-linking. It is also involved in intracellular copper transport (Pedersen *et al.,* 1996). GSH can chelate copper ions and diminish their ability to generate free radicals, or atleast to release radicals into solution (Hanna and Mason, 1992). GSH is a radioprotective agent and a

cofactor for several enzymes in different metrabolic pathways, including glyoxylases (Thornalley, 1996) and enzymes involved in leukotriene synthesis.

Glutathione also plays a role in protein folding and the degradation of proteins with disulphide bonds, such as insulin (the first step in insulin removal is cleavage of disulphide bridges linking the two peptide chains (Bellis *et al.,* 1994). Glutathione is also involved in the metabolism of herbicides, pesticides and *Xenobiotics* ('foreign compounds') generally in both animal and plant tissues (Hayes and Pulford, 1995).

Many xenobiotics supplied to living organisms are metabolized by conjugation with GSH, catalysed by glutathione S-transferase (GST) enzymes.

It is much more difficult to a deplete mitochondrial GSH than cytosolic GSH. Very severe GSH depletion in animals leads to widespread mitochondrial damage

(e.g. to Kidney, liver, lung, brain and eye) (Meister, 1995). Tissue injury is ameliorated by replacing GSH, e.g. with a GSH 'delivery agent' such as GSH ethyl or methyl esters. Mitochondria cannot synthesize GSH and must absorb it form the cytoplasm through an inner membrane transporter.

Glutathione peroxidases (GPX) / detoxify various peroxides using the reduced form of glutathione (GSH) as an electron donor and constitute a large family of groups (Brigelius-Flohe, 1999). These enzymes are classified into two groups in terms of the active site amino acid one of which contains selenocysteine (sec) at its active center, while the other does not. Since selenium (se) deficiency is related to male infertility, the relationship between GPX activity and male fertility has been debated (Hansen and Deguchi, 1996). At least four isozymes belong to selenium-containing GPX in mammals. The cytisolic form, GPX 1 is widely distributed in tissues and has been most extensively investigated (Fujii *et al.,* 2002). GPX 1, like other antoxidative enzymes, prevents apoptosis induced by oxidative stress and other stimuli (Kayanoki *et al.,* 1996). GPX 2 and GPX 3 are gastrointestinal and plasma forms, respectively, and number of studies on then regarding the reproductive process have appeared. GPX is a non-selenium enzyme and, hence, should be classified in to the non selenium-development GPX group. Because it is named so and is highly associated with the male reproductive system. GPX is expressed exclusively in the epididymis (Hall *et al.,* 1998) and is secreted and present in the caput and cauda epididymides lumens. (Rejraji *et al.,* 2002). The binding of GPX 5 to sperm membrane has also been reported. Thus, the protection of the sperm membrane against peroxidation is a possible function of this epididymis-specific is form (Vernet *et al.,* 1999). Since selenium deficiency causes male infertility and sec-containing GPX is suspected to be a candidate for the defective molecule non-selenium type GPX 5 is speculated to serve as the back up enzyme for the sec-containing GPX. The activity of non selenium dependent GPX is low and, hence, its contribution as a GSH-dependent peroxide scavenger is ambiguous.

In the present study, there was an increase in lipid peroxidation process and lowered glutathione concentration after ciprofloxain treatment at both low and high doses.

The lowered glutathione suggests an inhibition if glutathione induced cell protection against oxidative damage and membrane lipid peroxidation. Co-administration with vitamin's C, E, and A had a protective effect on glutathione levels which were raised above the drug induced decrease. This observation suggests the expression of anti-oxidant effects of these vitamins. Vitamin A appear to be more effective in this action compared to others.

The decrease in reduced glutathione, an endogenous antioxidant, levels in azoospermic and oligozoospermic conditions may cause disruption in the membrane integrity of spermazoa as a consequence of increased oxidative stress. Co-administration with vitamin C, E and A protects against the ROS mediated damage on spermatozoa. Vitamin E supplementation could be of clinical importance for prolonged spermatozoal storage whenever needed (Verma and Kanwar, 1999). Co-administration with vitamin C had protective effect against bisphenol A-induced toxicity in epididymal sperm and epididymis (Chitra *et al.,* 2003)

Many drugs are known to adversely affect the testicular spermatozoa production of spermatogenesis and spermiogenesis. Milton Prabu, (1997) has shown cadmium chloride induced oxidative stress and decreases in antioxidant protein, the glutathione. Chitra *et al.* (2003) have shown Bisphenol A administration to cause increase in oxidative stress in epididymis and to cause degeneration of the epididymal epithelium of rats. Bhardwaj *et al.* (2000) have shown the decrease in glutathione, an endogenous antioxidant, levels in azoospermic and oligozoospermic conditions cause disruption in the membrane integrity of spermatozoa as a consequence of increased oxidative stress.

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