

The Protective Effect of Green Tea Extract against Enrofloxacin Action on the Rat Liver; Histological, Histochemical and Ultrastructural studies

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Abstract: The bioavailability of enrofloxacin (EFX) was determined after single intraperitoneal administration to healthy adult albino rats. The aim of this trial was to evaluate, on what extent, the different doses of the green tea extract (GTE) as an antioxidant encompass a protective effect on the toxicity of EFX. Consequently, the study was carried out in three groups as follows: group 1, control animals; group 2, rats medicated only with daily dose of 75mg/kg enrofloxacin for 10 days and group 3, rats receive daily dose of 75mg/kg enrofloxacin and green tea extract for the same period (10 days). The last group was divided into three subgroups; subgroup A, received EFX of the concluding dose plus 1% GTE, subgroup B, received EFX of the same dose plus 1.5% GTE and subgroup C, received EFX of the similar dose plus 3% GTE. After the experimental period, small pieces of the liver tissue were taken and prepared for purpose of the histological, histochemical and electron microscopical examination. The results revealed that enhancement of EFX produces sever alterations in the hepatic tissue. It ascribed disturbances in hepatic architecture besides liver cells appeared hypertrophy correlated with necrotic nuclei. Congested blood sinusoids with leucocytic infiltration were apparent. Hepatocytes induced poor glycogen storage and exhausted proteins. Ultrastructural study demonstrated scattered cytoplasmic organelles after the destructed cell membrane from the burst down of the cell. GTE supplementation partially repairs the toxic effect of EFX and ameliorates the hepatic tissue especially when consumed by higher doses. Cytoplasmic glycogen and protein come again too increased. The fine structure manifested more or less intact hepatocytes through restored organelles constituents especially numerous profiles of granular endoplasmic reticulum, few lysosomes, normal glycogen deposits, euchromatic nuclei and distinct nucleoli as well as few lipid droplets in the cytoplasm. It was concluded that GTE is an important appropriate anti-oxidant improving the EFX toxicity at the altitude of the different doses however more improvement was observed after the consumption of higher ones.

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1. Introduction:

Enrofloxacin a fluorinated quinolone carboxylic acid derivative is a chemotherapeutic agent use in human and veterinary medicine (Gorla et al., 1999). Its activity as antibacterial drugs against aerobic and anaerobic bacteria is essentially due to the effective inhibition of DNA replication by binding and inhibiting its synthesis (Elmas et al., 2001; Turel, 2002). Besides, Vaccaro et al. (2003) reported that the EFX seems to be inhibitor to cytochrome P450. Furthermore, quinolones were found to induce singlet oxygen and superoxide anion (Abd-Allah et al., 2000). It was demonstrated that the bacteriostatic activity of the tetracyclines at which enrofloxacin belonged is associated with inhibition of protein synthesis that is to be dependant on the time and the drug concentrations (Scholar and Pratt, 2000). The mechanism of its activity is preventing DNA supercoiling and decantation of original chromosomes to replicates (Turel, 2002). Moreover, it believed that in the presence of the magnesium metal ion, interact efficiently with gyrase

or the gyrase-DNA complex leads to an unstable condensation of the DNA configuration of the bacterial DNA molecule during cell division (Lecomte et al., 1998; Sissia et al., 1998; Michiels et al., 2002; Noble and Maxwell, 2002 ; Xu et al., 2006).

Due to the potential antioxidant of green tea that is far greater than of vitamin E and/or C (Wiseman, 1997 and Rice-Evans, 1999), a number of different studies have been attributed including prevention liver and kidney injury induced by drugs and toxic agents. It improve the hepatic metabolism and intestinal absorption and cancer prevention (Yan et al., 2006; Walle, 2007), used for ameliorates acute lung inflammation after exposure to cigarette smoke (Lanzetti et al., 2008; Chan et al., 2009) and improve fenitrothion insecticide toxicity (Elhalwagy et al., 2008). However, several repots have been suggests that catechins is responsible for the most effective property in the inhibition of lipid peroxidation (Ishikawa et al., 1997) and it react with peroxyradicals in phospholipids

bilayers via a single electron transfer followed by deprotonation (Javanovic et al., 1996).

The aim of the present work is to evaluate the effects of green tea extract against toxicity induced by antimicrobial agent, enrofloxacin 10%. Green tea has been attributed as a potent anti-oxidant, so we thought to study the improvement occur in the liver after consuming of different doses of the extract. It was investigated by means of histological, histochemical and ultrastructural methods.

2. Material and Methods

1- Chemicals and drugs:

China green tea (Kangra, Himanchal Pradesh, India and Lipton-Unilever, Englewood Cliffs, NJ, USA) of commercially available market was purchased locally packaged by the Egypt National Native Product sources. Enrofloxacin 10% manufactured and purchased from the local company; El-Nasr pharmaceutical chemicals Co., Egypt.

2- Preparation of green tea:

Green tea extract was freshly prepared everyday by brewing (25-30 gm) dried tealeaves in 500 ml of boiling water then cooled to room temperature. Tealeaves solution was filtered and extracted second time with 500 ml of boiling water then filtered. The two filtrates were combined to obtain 3% green tea extract (3g tealeaves /100 ml water), in that case, it poured into the animals' feeding bottles. 1.5 % and 1% green tea extract prepared by the identical method (Khan et al, 2009). The resulting clear solution is similar to tea consumed by human. All animals were given a solution of green tea extract instead of drinking water.

3- Animals and experimental design:

Adult male rats (*Rattus norvegicus*), (3–4) months age, weighing 100 -120 g, obtained from the National Research Center in Dukki, Cairo (N.R.C.) were placed in wire mesh cages and housed in usual situation temperature ($25 \pm 2^{\circ}\text{C}$) with a relative humidity and a 12h light/ dark cycle. All animals were acclimatized for 1 week before the experiment, fed natural diet and given fresh water ad libitum. The animals were randomly divided into groups (5 per group) and kept in separate cages.

Group 1: (Control) animals received no specific treatment.

Group 2: Animals were injected intraperitoneally with enrofloxacin 10% (75 mg/kg B. W.) daily for 10 days to provoke the damage effects on the liver.

Group 3: Animals were injected intraperitoneally with enrofloxacin10% (75 mg/kg B. W) daily for 10 days and feed with green tea extract to identify its

protective effects on the liver. This group was divided into three subgroups as following:

Subgroup A: Animals were injected intraperitoneally with enrofloxacin10% (75 mg/kg B. W) daily and feed with 1% green tea extract in stead of drinking water.

Subgroup B: Animals were injected intraperitoneally with enrofloxacin10% (75mg/kg B. W) daily and feed with 1.5 % green tea extract in stead of drinking water.

Subgroup C: Animals were injected intraperitoneally enrofloxacin10% (75 mg/kg B. W) daily and feed with 3% green tea extract in stead of drinking water.

Histological study:

After rats were killed small pieces of liver were removed and fixed by means of 10 % buffered neutral formalin. The specimens were dehydrated and cleared afterward embedded in paraffin blocks. Paraffin sections were cut with at 5μ thickness and stained with routine hematoxylin and eosin (H &E) stain and Crossmon's trichrome technique for evaluating the collagen fibrosis.

Histochemical study:

In parallel, histochemical finding was established by means of PAS reaction for glycogen stored and bromophenol blue method for detection of proteins in both controls the treated animals (Bancroft and Stevens, 1990).

Ultrastructural study:

Small pieces of liver were fixed in 2% glutaraldehyde fixative in 0.1M Na- cacodylate buffer, pH 7.2, followed by three washes in the buffer, then post fixed during 1 h in 1% OsO₄ in the same buffer, dehydrated, cleared and infiltrated in Resin. Ultrathin sections were stained with 2% uranyl acetate, followed by Reynold's lead citrate stain and examined with a Siemens ELMISKOP I or Zeiss M-109 Turbo electron microscope.

3. Results

In control rat liver: the hepatic tissue appeared normal microscopically, the central vein from its region, the hepatic cords were radiating and separated by the blood sinusoids (S) (Fig.1). The distribution of proteins in the hepatocytes (in faint blue colorations) was demarcated (Fig. 2).

The ultrastructural picture of the hepatic tissue in control rat liver revealed, normal hepatic cell euchromatic nucleus (N) with two normal nucleoli (n), numerous intact mitochondria(m), Golgi apparatus(G), granular endoplasmic reticulum profiles(ER), hepatocyte intact cell membrane(mem), primary lysosomes and glycogen deposits (Fig.3).

Liver tissue after daily dose of enrofloxacin 10% and sacrifice after 10 days: The hepatic tissue revealed severe ballooning degeneration of many hepatocytes and some of them were burst down and destructed or necrotic, central vein with perivenous leucocytic infiltration and congested blood sinusoids (Fig.4). After Crossmon's trichrome staining there was traces of collagenic stroma, congested blood vessels including the sinusoids, severe ballooning degeneration of the hepatocytes, that some of them were necrotic or destructed (Fig.5). A common vacuolization along with the hepatocytes swelling and ballooning due to hydropic degeneration and progress to focal necrosis in the course of the hepatic lobules and some degree of hepatic hyperatrophy. The liver showed established acute inflammatory cells and exudates were increased in these animals intoxicated with enrofloxacin.

No marked PAS +ve reaction in the hepatocytes due to deprived glycogen, a number of hepatocytes suffered from severe ballooning degeneration, others were destructed with pervascular leucocytic infiltration (Fig.6) in comparison to the control that showed no congestion in blood vessels, no leucocytic infiltration, normal distribution of glycogen (PAS +ve magenta colour) through the hepatocytes (Fig.7).

A depleted protein was induced from the hepatocyte cytoplasm even in some of the nucleoprotein of the hepatocyte nuclei (Fig.8) to be compared with the control of Fig. 2.

The electron microscopic picture exhibited ruptured hepatocyte after severe ballooning degeneration, and so its nucleus. A haemorrhagic area with oozing blood red blood corpuscles, scattered cytoplasmic organelles after the destructed cell membrane of the burst down of the cell was in attendance, (Figs.9 & 10).

So, it is likely to say that EFX given dose caused severe hepatic toxicity that was reflected by the all hazards manifested on the hepatocyte and hepatic blood stagnation in the liver tissue.

Liver tissue of rats after daily dose of enrofloxacin 10% and 1% green tea extract: The hepatic tissue showed no congestion of the blood sinusoids, many normal intact hepatocytes, less abundant leucocytic infiltration (Fig.11). After Crossmon's trichrome staining, there were few collagen fibril deposition (in green) in the hepatic stroma, (Fig.12), the hepatocytes took the same description of Fig.11.

Many hepatocytes deposit glycogen in their cytoplasm (magenta colour), (Fig.13).

By electron microscopy, there were intact hepatocytes with many normal mitochondria, slightly decreased granular endoplasmic reticulum profiles,

intact euchromatic hepatocytic nucleus with normal nucleolus, few primary lysosomes, normal Golgi complex few lipid droplets (p) and glycogen deposits, (Fig.14).

Liver tissue of rats after daily dose of enrofloxacin 10% and 1.5% green tea extract: The hepatic tissue appeared with no congestion of blood sinusoids, many normal intact hepatocytes, and prominent active von Kupffer cells in the blood sinusoids (Fig.15). The nuclei of the hepatocytes were more prominent clear with clearance of their nuclei. Little differences between it and that of 1% treatment were common.

Most of the hepatocytes revealed PAS +ve glycogen deposition (in magenta colour), no congestion of blood vessels including the sinusoids and very few numbers of necrotic hepatocytes, due to ameliorating effect of green tea extract (Fig.16).

The electron microscopic picture exhibited hepatocytes with intact cell membrane, many normal intact mitochondria, few necrotic or destroyed hepatocytes, euchromatic nuclei of normal hepatocytes, normal granular endoplasmic reticulum profiles and glycogen deposits, with few lipid droplets (Fig.17).

Liver tissue of rats after daily dose of enrofloxacin 10% and 3% green tea extract: The hepatic tissue demonstrated that most of the hepatocytes to be normal with vesicular nuclei and normal nucleoli, no vascular including sinusoidal congestion, no leucocytic infiltration (Fig.18). It was verified that the cytoplasm became more eosinophilic and the nuclei became more prominent with recovered nuclei i.e, they were improved in comparison to the enrofloxacin given rats. It was noticed also that there is still necrotic cells present and slight vacuolation compared to the enrofloxacin alone. There were no differences between the normal control and green tea extract given groups.

Many of the hepatocytes deposited intracytoplasmic glycogen (Magenta colour), but few ones were not (Fig.19). The protein content of the hepatocytes nearly was restored to be more or less as those of control (Fig. 20).

With the electron microscopic manifestation there were more or less intact hepatocytes with restored organelles' constituents especially numerous profiles of granular endoplasmic reticulum, few lysosomes, normal glycogen deposits, euchromatic nuclei and distinct nucleoli as well as few lipid droplets in the cytoplasm of some hepatocytes (Fig.21) as those of controls, no expanded blood sinusoids (Fig.22). Those improved picture of the hepatic tissue is suggested to be due to the amelioration effect of green tea extracts at those short periods.

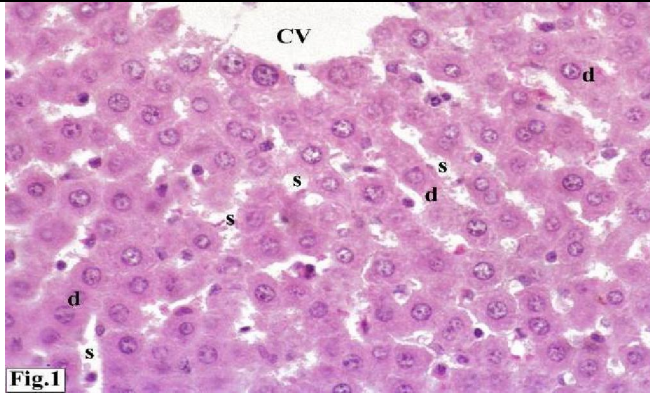


Fig.1: Photomicrograph for control rat liver displaying the central vein (C), hepatic cords (d) and blood sinusoids (S). H&E stain, X400.

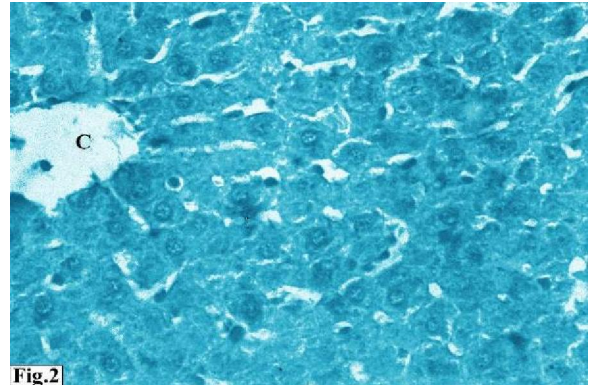


Fig.2: Photomicrograph for control rat liver distribution of proteins in the hepatocytes (in faint blue colouration), central vein(c), hepatic cords separated by blood sinusoids. Bromophenol blue stain, X 400.

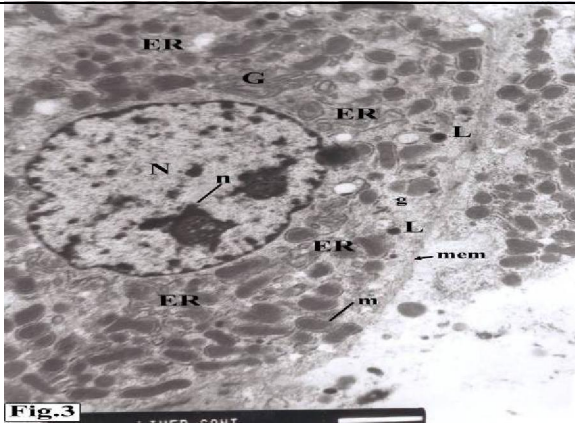


Fig.3:Electron micrograph for a control liver of rat, displaying, normal hepatic cell euchromatic nucleus (N) with two normal nucleoli (n), numerous intact mitochondria(m), Golgi apparatus(G), granular endoplasmic reticulum profiles(ER), hepatocyte intact cell membrane (mem), primary lysosomes, glycogen deposits(g), X 8000.

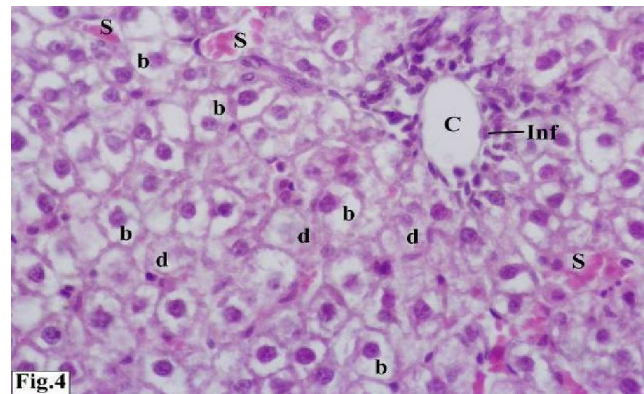


Fig.4: Photomicrograph for a section in the liver of rat administered daily dose of enro-floxacin 10% (75 mg/Kg B.W.) and sacrificed after 10 days of injection, displaying severe ballooning degeneration of many hepatocytes (b) and some of them were burst down and destroyed or necrotic (d), central vein (c) with perivascular leucocytic infiltration (Inf), congested blood sinusoids (s). H & E stain, X400.

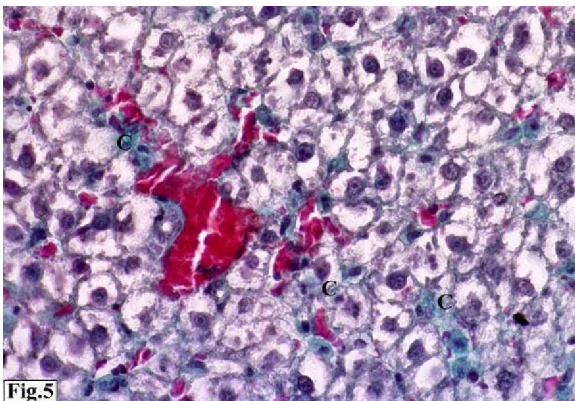


Fig.5: Photomicrograph for a section in the liver of rat administered daily dose of enro-floxacin 10 % (75 mg/Kg B.W.) and sacrificed after 10 days of injection, displaying stroma (c), congested blood vessels including the sinusoids, severe ballooning degeneration of the hepatocytes, that some of them were necrotic or destroyed as in Fig.3, Crossman's trichrome stain, X400.

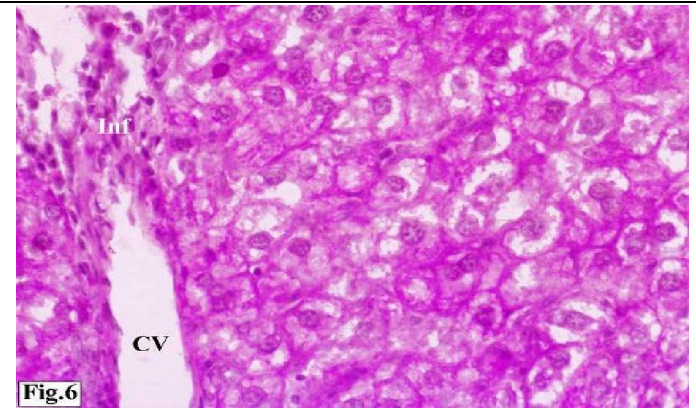


Fig.6: Photomicrograph for a section in the liver of rat, administered daily dose of enro-floxacin 10% (75 mg/Kg B.W.) and sacrificed after 10 days of injection, depicted no marked PAS +ve reaction in the hepatocytes due to deprived of glycogen, numerous of the hepatocytes suffered from severe ballooning degeneration, others were destroyed, perivascular leucocytic infiltration, PAS technique, X400

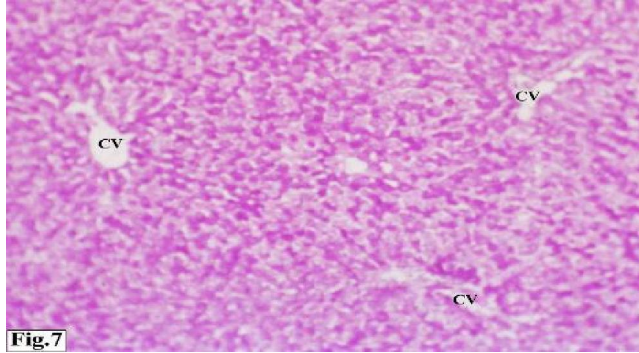


Fig.7

Fig.7: Photomicrograph for a section in a control rat liver for comparison of glycogen distribution in the hepatocytes with those of Fig.6, displaying no congestion in blood vessels, no leucocytic infiltration, normal distribution of glycogen (PAS +ve magenta colour) through the hepatocytes, PAS technique, X100.

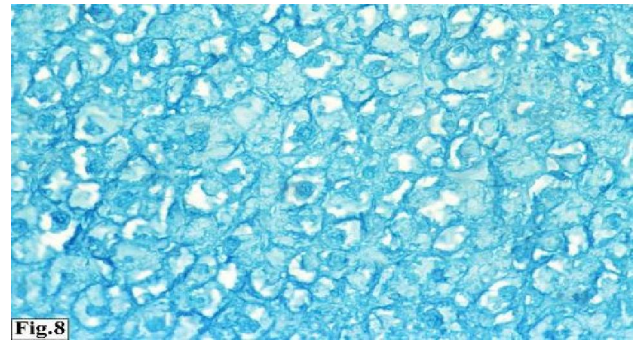


Fig.8

Fig.8: Photomicrograph for a section of rat liver administered daily dose of enro-floxacin 10 % (75 mg/Kg B. W.) and sacrificed after 10 days of injection, displaying depleted proteins from the hepatocyte cytoplasm even some of the nucleoprotein of the hepatocyte nuclei., to be compared with the control of fig. 2. Bromophenol blue stain, X400.

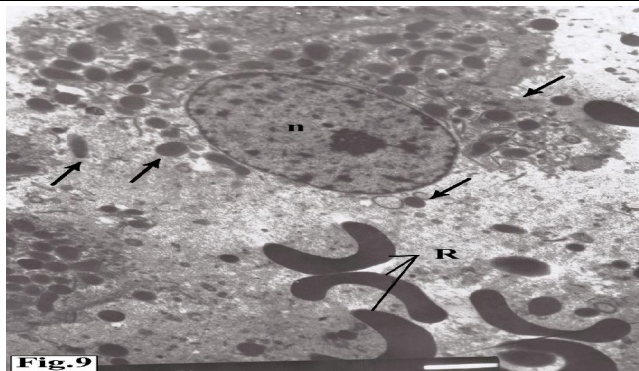


Fig.9

Fig.9: Electron micrograph for a section in the rat liver administered daily dose of enrofloxacin 10 % (75 mg/Kg B.W.) and sacrificed after 10 days of injection, displaying ruptured hepatocyte after severe ballooning degeneration, its nucleus (N), haemorrhagic area with oozing blood red blood corpuscles (R), scattered cytoplasmic organelles (arrows) after the destructed cell membrane after the burst down of the cell, X 6000.

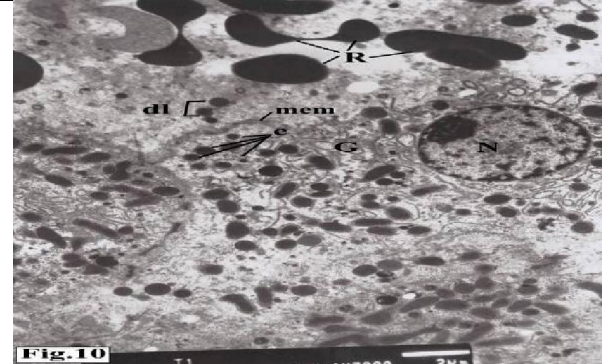


Fig.10

Fig.10: Electron micrograph for a section in the rat liver administered daily dose of enrofloxacin 10 % (75 mg/Kg. B.W.) and sacrificed after 10 days of injection ,displaying haemorrhagic spot with oozing red blood corpuscles (R) destructed cell membrane (mem) of burst down hepatocyte, abnormal hepatocyte configuration, discarded lysosomes (dl) Golgi app. configuration (G), atrophied cell nucleus (N), X 6000.

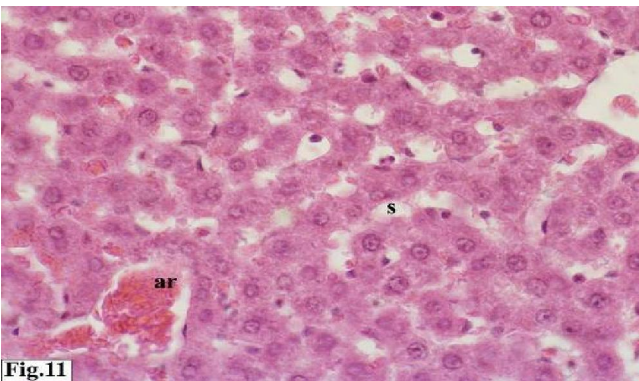


Fig.11

Fig.11: Photomicrograph for a section in the rat liver administered enrofloxacin 10% as daily dose as well as green tea extract(1%) instead of drinking water for continuous 10 days, then the rats were sacrificed, displaying, no congestion of the blood sinusoids(S), many normal intact hepatocytes , less abundant leucocytic infiltration. H & E stain, X400.

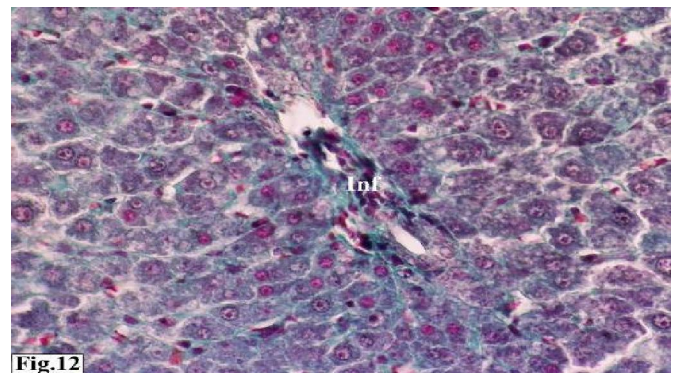


Fig.12

Fig.12: Photomicrograph for a section in the rat liver administered enro-floxacin 10% as daily dose as well as green tea extract(1%) instead of drinking water for continuous 10 days, then the rats were sacrificed, displaying few collagen fibril deposition (in green) in the hepatic stroma, the hepatocytes took the same description of Fig.11, Crossman's trichrome stain, X400.

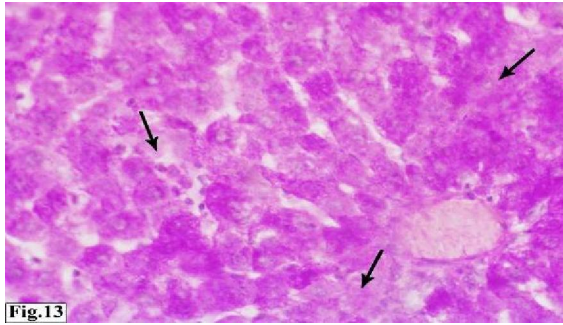


Fig.13

Fig.13: Photomicrograph for a section in the rat liver administered enrofloxacin 10% as daily dose as well as green tea extract (1%) instead of drinking water for continuous 10 days, then the rats were sacrificed, displaying no congestion in the hepatic sinusoids, less leucocytic infiltration in the portal tract, few necrotic or degenerated hepatocytes (arrows), many hepatocytes deposit glycogen in their cytoplasm (magenta colour). PAS technique, X400.

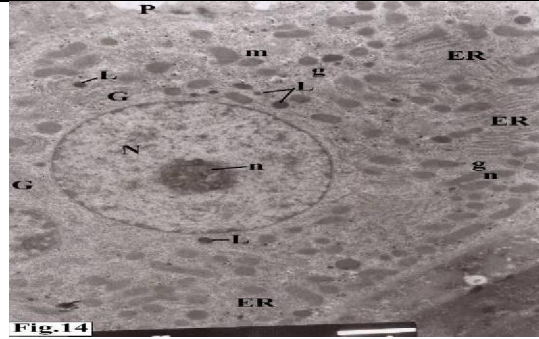


Fig.14

Fig.14: Electron micrograph for a section in the liver of rat administered daily dose of enrofloxacin 10% (75 mg /Kg B.W.) as one dose as well as green tea extract 1% instead of drinking water for continuous 10 days, then the rats were sacrificed, displaying, intact hepatocyte with many normal mitochondria (m), slightly decreased granular endoplasmic reticulum (ER) profiles, intact euchromatic hepatocytic nucleus (N) with normal nucleolus (n), few primary lysosomes (l), Golgi complex (G), few lipid droplets (p), glycogen deposits (g), X8000.

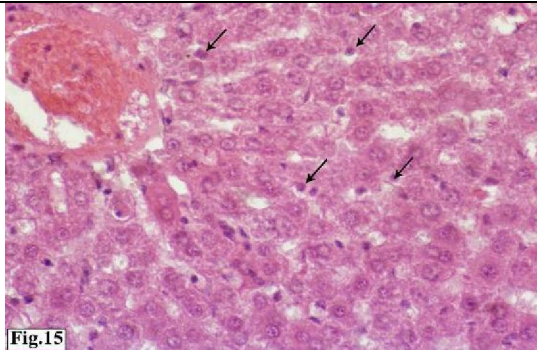


Fig.15

Fig.15: Photomicrograph for a section in the rat liver administered daily dose of enrofloxacin 10%(75 mg/Kg B.W.) as well as green tea extract 1.5% instead of drinking water for continuous 10 days, then the rats were sacrificed, displaying, no congestion of blood sinusoids, many normal intact hepatocytes, few number of degenerated hepatocytes, prominent active von Kupfer cells (arrows) in the blood sinusoids. H & E stain, X400.

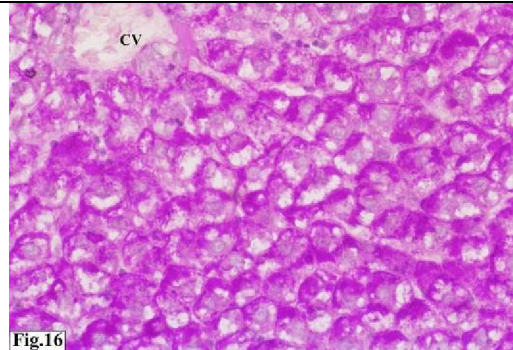


Fig.16

Fig.16 Photomicrograph for a section in the rat liver administered daily dose of enrofloxacin 10%(75 mg/Kg B.W.) as well as green tea extract 1.5% instead of drinking water for continuous 10 days, then the rats were sacrificed, concealed most of the hepatocytes revealed PAS +ve glycogen deposition (in magenta colour), no congestion of blood vessels including the sinusoids, very few number of necrotic hepatocytes, due to ameliorating effect of green tea extract. PAS-technique, X400.

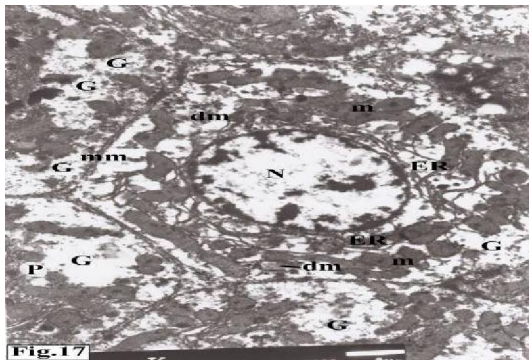


Fig.17

Fig.17: Electron micrograph for a section in the liver of rat administered daily dose of enrofloxacin 10 %(75 mg/Kg B.W.) as one dose as well as green tea extract 3% instead of drinking water for continuous 10 days, then the rats were sacrificed, displaying hepatocytes with intact cell membrane (mem), many normal intact mitochondria(m), few necrotic or destroyed hepatocytes, euchromatic nuclei (N)of normal hepatocytes, granular endoplasmic reticulum (ER), glycogen deposits(G), few lipid droplets(P), X6000.

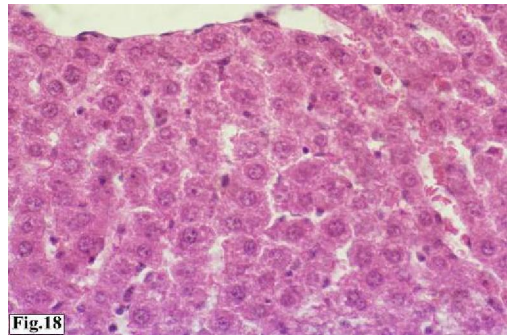


Fig.18

Fig.18: Photomicrograph for a section in the rat liver administered daily dose of enrofloxacin 10 %(75 mg/Kg B.W.) as one dose as well as green tea extract 3% instead of drinking water for continuous 15 days, then the rats were sacrificed, displaying, most of the hepatocytes revealed normal picture with vesicular nuclei and normal nucleoli, no vascular including sinusoidal congestion, no leucocytic infiltration, H & E st.,X400

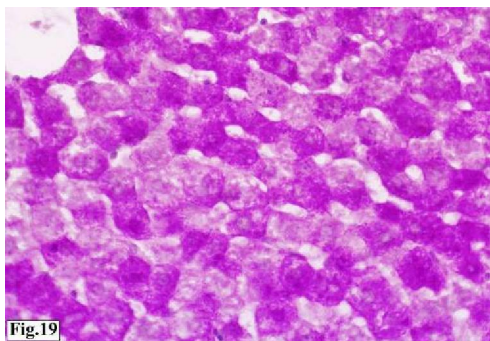


Fig.19

Fig.19: Photomicrograph for a section in the rat liver administered daily dose of enrofloxacin 10% (75 mg/Kg B.W.) as one dose as well as green tea extract 3% instead of drinking water for continuous 15 days, then the rats were sacrificed, displaying, many of the hepatocytes deposited intracytoplasmic glycogen deposition (Magenta colour), but few ones were not, no sinusoidal congestion, no leucocytic infiltration. PAS technique, X 400.

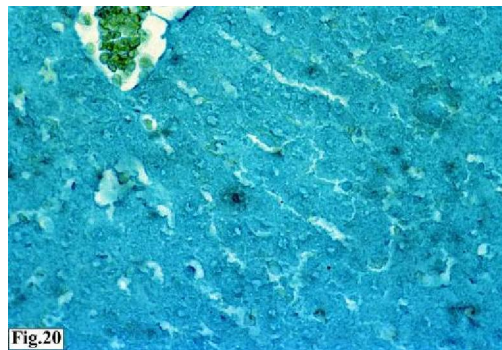


Fig.20

Fig.20: Photomicrograph for a section in the rat liver administered daily dose of enrofloxacin 10% (75 mg/Kg B.W.) as well as green tea extract 3% instead of drinking water for continuous 15 days, then the rats were sacrificed, displaying, restored proteins in the hepatocyte cytoplasm to be more or less as the control (Fig.2). Bromophenol blue, X400

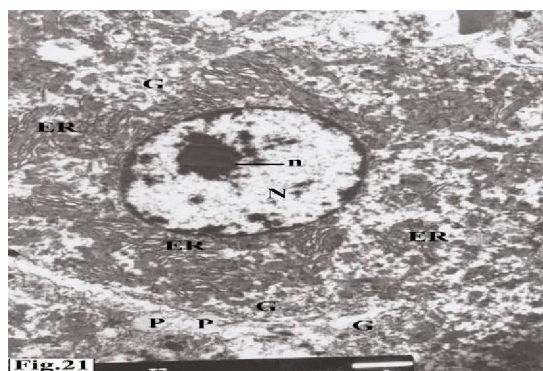


Fig.21

Fig.21: Electron micrograph for a section in the rat liver administered daily dose of enrofloxacin 10% (75 mg/Kg B.W.) as well as green tea extract 3% instead of drinking water for continuous 15 days, then the rats were sacrificed, displaying more or less intact hepatocytes with restored organelles constituents especially numerous profiles of granular endoplasmic reticulum (ER), few lysosomes (L), glycogen deposits (G), euchromatic nucleus (N) and distinct nucleolus (n) few lipid droplets in the cytoplasm of some hepatocytes as those of controls, X6000.

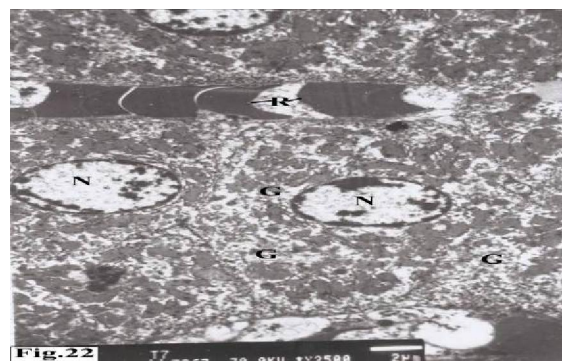


Fig.22

Fig.22: Electron micrograph for a section in the rat liver administered daily dose of enrofloxacin 10% (75 mg/Kg B.W.) as well as green tea extract 3% instead of drinking water for continuous 15 days, then the rats were sacrificed, displaying nearly normal hepatocytes after amelioration by the 3% green tea extract, tangential section of euchromatic hepatocytic nuclei (N), normal presence of red blood corpuscles (R) in non-expanded blood sinusoids, normal glycogen deposits (G), X5000.

4. Discussion

Enrofloxacin is an excellent bactericidal agent against broad spectrum of aerobic and some facultative anaerobic bacteria (Elmas et al., 2001). Oxidative stress may be common factor in liver diseases of different etiologies (Parola and Robino, 2001). However, it may be thought that the major cause of EFX-induced liver toxicity is inhibition of hepatic cytochrome P450 (Vaccaro et al., 2003) that responsible for the metabolism of the drugs. Tea, one of the most accepted beverages consumed world wide by man has traditional great interest concerning its possible contribution in hindrance of many diseases as well as antioxidant properties (Mukhtar and Ahmad, 2000). Green tea found to be health benefits associated with neuroprotective, antidiabetic and antibacterial (Alschuler 1998; Liao, 2001). Besides, it has markedly protective against many toxic agents as

CCl_4 -induces hepatotoxicity in rats (Zhen, 2007). The present study aimed at the investigation of the protective action of GTE concerning high content of EGCG, a major polyphenol in alleviating the adverse toxic effects of EFX.

A hepatotoxic dose of EFX was administered to control rat and GTE-consuming rats and the specific metabolic activities of liver were examined to ascertain potential advantage property of GTE. As shown in the results, EFX administration to rats created liver toxicity which was manifested by marked architectural disturbances of hepatic lobules as well as severe ballooning degeneration and hepatic necrosis, rising of the leucocytic infiltrations with congested blood vessels and increased collagen fibers. The increase of inflammatory cell of leucocytic infiltration might be powerful allies in body's defense against EFX-induced tissue destruction and hepatic

necrosis at which scavenger macrophages engulf dead cells as previously reported by Cotran (1999). EFX may accompanied by increase the cytosolic Ca^{2+} , by oxidative stress, by break down of phospholipid (Kumar, 2005) chief to this hepatic disturbances.

The present data agreed with work by Elasmag (2010) who found severe hepatic necrosis and DNA damage in liver kidney and spleen following administration of the identical dose of EFX, in addition to genotoxic results observed by Gorla et al. (1999). besides, histological degeneration of the testicular tissues and sperm abnormalities and morphology in male mice induced by administration of EFX (Aral et al., 2008).

After feeding with lower doses of green tea extract slight ameliorations of the unfavorable effects produced in the liver by EFX activity, which was associated with less leucocytic infiltration, glycogen enhancement in the cytoplasm, many normal intact hepatocytic nucleus and nucleolus, many normal intact mitochondria, few necrotic or destroyed hepatocytes, euchromatic nuclei of few normal hepatocytes with few lipid droplets. GT probably arrest the harmful mechanism of liver injury through protection of cells and tissues from oxidative damage by scavenging oxygen-free radicals and stimulate the regeneration of damaged tissues and cells (Jimenez-Lopez and Cederbaum, 2004; Feng et al., 2001). The result confirmed by many authors (Alschuler, 1998; Liao, 2001; Fadhell and Amran, 2002; Khan and Mukhtar, 2007), they stated that consumption of black tea and green tea has many beneficial effects on human health, particularly polyphenols, chiefly catechins and their derivatives that retard various forms of cancers due to its antimutagenic, anticarcinogenic and antioxidant properties, cardioprotective, neuroprotective, antidiabetic and antibacterial.

EFX injection caused depletion of glycogen stores in the hepatocytes indicating damaged induced in the liver tissue. It possibly due to the increased rate of anaerobic glycolysis maintaining cell energy as well as oxygen deficiency and consequently decreased many cellular enzymes activities induced by the drug (Kumar et al., 2005).

The present data of lower doses GTE consumption to EFX-treated rats result an overall improvement of carbohydrate metabolism indicating by increase of glycogen storing to the hepatocytes but in a less extent i.e. higher doses best ameliorate glycogen hepatocytes. In this respect, Waltner-law et al. (2002) explain the lowered blood glucose to the decrease in expression of genes control gluconeogenesis in liver cells while Khan et al. (2007) elucidated that GTE causes selective adaptive

alterations in the activities of certain mitochondrial enzymes involved in glycolysis, gluconeogenesis and glycogenesis in the liver cells. Also it has been shown to increase energy expenditure and fats oxidation in human (Dulloo et al., 1999; Diepvens et al., 2005).

Concerning histochemical demonstration of protein disruption after EFX treatment might be due to detachment of ribosomes from rough endoplasmic reticulum and hence reduction of protein syntheses corresponded to stress of the drug which cause damaged enzymes and free radical (Karmar et al., 2005). Moreover, EFX may cause increase in cytosol Ca^{2+} that mediates variety of deleterious effects activates a number of enzymes cell death which cause membrane damage, protein and DNA and chromatin fragmentation. In contrast to GTE consumption in combination with EFX treatment resulted reversal alteration in various protein activities in the liver tissue compared to EFX treated liver. Many hepatocytes deposited intracytoplasmic glycogen but few ones were not and the protein content of the hepatocytes nearly was restored to be more or less as those of control. The elevation of protein synthesis and hence glycogen may explain by green tea polyphenols that stimulate the transcription of phase II detoxifying enzymes mediated by an antioxidant response element (Ranjbar et al., 2005). It is probable due to the downregulation by the green tea as denoted by McCarthy et al. (2007) who found that green tea catechin suppressed the cellular DNA replication and reduces protein gene expression. In the other hand, it was previously reported that GT activities prevent damage to DNA structure (Hider et al., 2001).

Histological and histochemical disturbance occurred in liver tissue were confirmed by electron microscopic changes in liver. Hence, after EFX treatment of liver it revealed rupture of hepatocytes, haemorrhage, and scattered cytoplasmic organelles after the destruction of cell membrane. This deterioration may be regarding to the cytotoxic effects of EFX that may cause an increase in cytosolic calcium concentration owing to the net influx of Ca^{2+} across the plasma membrane and release of Ca^{2+} from mitochondria (Kumar, 2005). Or may be due to the presence of the acidic and basic functional groups of enrofloxacin giving it lipid-soluble compounds and can be penetrate tissues (Brown, 1996; Gorla et al., 1999; Abo-Elsood, 2003). Meanwhile, inhibition of catalase activity and a lipophilicity property of EFX confirm the potential bioaccumulation owing to this toxic effects was reported by Gao et al. (2008) and Migaliore et al. (2003). Our results were confirmed by previous study (Coetzee et al., 2009) that showed a significant morphological and ultrastructural

changes following treatment of enrofloxacin to *Anaplasma marginale*.

Destruction of intracellular organelles such as mitochondria, rER, lysosomes reflect the major structural and functional integrity of the intracellular assessed by the status of their respective biomarker enzymes (Khan et al., 2009).

GTE might cause lowered number of damaged mitochondria or affected macromolecules or may increased number of normally active organelles or macromolecules a marked increase in glycogen, protein, enhance lipids appears to be

Higher dose (3%)GTE showed an improvement of the cell membranes destructed by EFX and so cytoplasmic organelles was noted intact cell membranes of the hepatocytes, in this regard, Ostrowska et al.,(2004) found that enhancement in lipid peroxidation was associated with disruption of hepatocyte cell membranes, as observed through electron microscopic evaluation. So Green tea protects phospholipids from better peroxidation and prevents changes in biochemical parameters and morphologic changes. Hence, the results of this work support the suggestion that green tea protects membranes from peroxidation of lipids associated with ethanol consumption in rat liver by decreasing oxidative stress (Augustyniak, et al., 2005)

Chemopreventive intervention by different phytochemicals, particularly tea polyphenols found in green tea show 20 times more powerful antioxidant activity than vitamin C (Craig 1999). It enhances expression of intracellular endogenous antioxidants such as glutathione, glutathione peroxidase, glutathione peroxidase catalase (Khan et al., 1992; Valerio et al., 2001) reported that it reduces the generation of reactive radicals. Green tea extracts act as an antioxidant both intracellularly and extracellularly in conjunction with various enzymatic processes, multiple intracellular functions including detoxification of reactive oxygen intermediates and reduction of low-molecular weight thiols and sulfides and mixed disulfides of proteins (Ookhtens M., 1998).

In conclusion, the observation of the current study demonstrated that EFX induces production of free radical that causes oxidative scratch to the liver cells and its organelles particularly mitochondria and cell membranes. Green tea reduces this oxidative damage by its antioxidant properties and ameliorates against the drug- induced hepatotoxicity

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