Biochemical and cytogenetic Investigations into the effects of
Enrofloxacin in rats

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Abstract

Oral administration of enrofloxacin at doses of 7.5 and 10mg for 30 successive
days into mature rats significantly increased the frequencies and percentage of micro
nucleated polychromatic erythrocytes (MPCEs) and ratio of polychromatic erythrocytes
normochrotic erythrocytes (PCE / NCE) was recorded.

The effect of enrofloxacin on body weight was studied in 2 groups of 10 mature
rats each was orally administered by the tested drug at a dose of 7.5 and 10 mg/
100gm.b.wt. for 30 successsiundays. Enrofloxacin showed insignificant increase in
bodyweight of the rats at the doses. The tested drug significantly increased the activities
of serum Aspartate amino transaminase(AST), Alanine amino transaminase (ALT) and
the level of urea, but decreased the alkaline phosphatase (AP) activity and level of total
protein

Introduction

Fluoroquinolones are anti-microbial agents, with broad spectrum of bacterial
activity against both gram-positive and gram negative bacteria. (Oliphant et al 2002 and
Shenoy et al 2011 ) They are bacterial agents that exert their bacterial action by
inhibiting the action of bacterial enzymes DNA gyrase (layrence and parker 2008)

Fluoroquinolones are well tolerated in patients but their uses have been
associated with some adverse effects (Wolfson and Hooper 1985 and Grayson ,1999). Enrofloxacin is a second generation fluoroquinolone antibacterial agent (Martinez et
al., 2006)

The effect if enrofloxacin on polychromacticand normochromatic cells seems to
be deficient and requires more investigation. Accordingly this work was conducted to
clarify the effect if enrofloxacin on polychromatic, normochromatic, body weight and
serum enzymatic activies and levels of total protein, creatinine and urea.

Material and Method

Enrofloxacin (1-cyclopropyl-7-(4-ethyl -1- piperazinyl)–b- Fluoro-1,04-dihydro-4-oxo-3-quinolone carboxylicacid) was produced by El Nasr pharmaceuticals chemicals
company, Egypt.
Animals Thirty mature rats of an average body weight 150-180 gm were used. Rats were fed on ordinary ration and water ad libitum.

I. Cytogenetic:

In order to investigate the possible mutagenic effects of enrofloxacin (in low and large dose), the micronucleus test was performed to detect chromosomal damage associated with the treatment. Micronuclei were identified as dark blue staining bodies in the cytoplasm of the poly chromatic erythrocytes (PCEs).

Animals were divided into, 3 groups of 10 mature rats each were used first was kept as a control, the second and third groups were orally administered enrofloxacin at doses 7.5 and 10 mg / 100g.b.w.t for 30 successive days (long duration).

Following the protocol established by Salamon et al., (1980), bone marrow cells of rats were extruded with a pin into a clean dry glass slides and homogenized with two drops of fetal calf serum. Cells were smeared on the slide, air dried, fixed in absolute methanol and stained with Giemsa in phosphate buffer pH 6.8. The poly chromatic erythrocytes (PCEs, 1000 / animal) were screened for micronuclei, and the changes in the mitotic activity (Hart and Engberg –Pederson, 1983; Al– Bekairi et al., 1991)were assessed on the basis of the ratio of polychromatic to normochromatic erythrocytes (PCE / NCE ratio).

II- Effect of enrofloxacin on body weight and biochemical analysis:

Rats of each group were weighted at the beginning of the experiment, then every week for 30 days. The changes in body weight was calculated and recorded.

Individual blood samples were obtained from rats, left to clot and sera were separated for biochemical analysis. The activities of AST, ALT and AP were determined by Reitman and Frankel (1957) and Roy (1970) while total protein, urea and creatinine were estimated as explained by Peters (1968), Kaplan (1965) and Husdan and Rapopart (1968).

The results were subsequently analyzed by following the statistical methods established by Snedcor (1969) in order to determine whether a dose group was positive or negative.

Result and Discussion

I. Cytogenetic:

The effect of enrofloxacin on the frequencies and percentage of micro nucleated polychromatic erythrocytes (MPCEs) as well as the ratio of PCE/NCE in control and treated animals are recorded in table (1).
Oral administration of enrofloxacin at 7.5 and 10mg/100 gm b.w.t. to mature rats for 30 days recorded significantly increased the percentages of MPCEs (1.71% and 2.07% respectively) when compared with corresponding control values. The PCE/ NCE ratio significantly increased of compared with control.

The significant increase in PCE/ NCE ratio according to Guyton (1991) and enhancement of mitotic activity of bone marrow cells could be considered as a sign of toxicity and /or damage of some organs of the body. Limited information is available about genetic activity of enrofloxacin (Zowail et al., 2009, Majed et al.,2015, Fatai et al., 2013 and Priyadharshini, 2013). Enrofloxacin at doses 7.5 and 10mg/100 gm.b.w.t affected seriously the examined body organs enough to enhance the production of PCEs in bone marrow, there by attempting to supply the demand for RBCs in the body. Our study, conducted by lebket al., (1982) found that plasmids mediated TR strains of staphylococcus auras was sensitive to enrofloxacin than other tetracycline and minocycline drugs.

Enrofloxacin is a second generation of fluroquiolone antibacterial agent (Martinez et al., 2006) against bacteria especially mycoplasma (Grayson 1999). However, the cytogenetic and biochemica l studies have not been investigated.

The effect of oral administration of enrofloxacin at doses of 7.5 and 10 mg /100gm. b.w.t. daily for 30 successive days on activities of AST, ALT and urea level induced significant increased but significantly decreased AP activity and level of total protein(table 2). Similar results were previously obtained by Fatai et al2013; priyadharshini2013;olusegun & Emmanuel 2014 and starling et al.,). The increase of AST and ALT activities reflects the degree of tissue damage. A good deal of researchers (Duncan and Prasse, 1981) claimed that the activity of AST increased in inflammatory and degenerative changes in liver because of increase liberation of enzymes from hepatic cells. In addition, concomitant administration of tested drug increased serum urea level which is specific indicator of renal damage (Rule et al., 2004).

On other hand, the level of total protein was significantly decreased (Fatai et al., 2013).

The increase in body weight after oral administration of enrofloxcin is in significant(table) which coincides with the study of Gamguihem et al., 2008 and Priyadharshini, 2013) who reported that administration of ciprofloxacin and did not affect growth of male rats.

Enrofloxacin had be proved to be mutagenic ,also it included degeneration in the visceral organs when used in repeated dose for a long period.
Table (1): Effect of enrofloxacin on the incidence of MPCE on the relation of PCE to NCE in rats mean ± S.E (n=10)

<table>
<thead>
<tr>
<th>G</th>
<th>Dose/mg 100g. b.wt.</th>
<th>PCE Screened</th>
<th>MPCE</th>
<th>No</th>
<th>NCE Screened</th>
<th>PCE/NCE Ratio</th>
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<tr>
<td>C</td>
<td>-</td>
<td>10000</td>
<td>56</td>
<td>11.2 ± 0.06</td>
<td>1.12</td>
<td>4366</td>
</tr>
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<tr>
<td>Enrofloxain</td>
<td>7.5</td>
<td>10000</td>
<td>86</td>
<td>17.2 ± 0.08***</td>
<td>171</td>
<td>2534</td>
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<tr>
<td>Enrofloxain</td>
<td>10</td>
<td>10000</td>
<td>104</td>
<td>20.7 ± 0.14</td>
<td>2.07</td>
<td>2784</td>
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</table>

Table (2): Showing oral administration of enrofloxacin on serum enzymatic activity and biochemical constituents in serum of rats. mean ± S.E (n=10)

<table>
<thead>
<tr>
<th>G</th>
<th>Dose/mg 100g. b.wt.</th>
<th>ALT U/L</th>
<th>AST U/L</th>
<th>AP U/L</th>
<th>Urea mg%</th>
<th>Creatinine mg%</th>
<th>Total protein gm%</th>
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<tr>
<td>C</td>
<td>-</td>
<td>3.58 ± 0.12</td>
<td>3.72 ± 0.13</td>
<td>12.96 ± 0.21</td>
<td>29.54 ± 0.16</td>
<td>0.53 ± 01.3</td>
<td>8.27 ± 0.21</td>
</tr>
<tr>
<td>Enrofloxain</td>
<td>7.5</td>
<td>4.90*** ± 0.12</td>
<td>4.7*** ± 0.17</td>
<td>11.27*** ± 0.29</td>
<td>25.86*** ± 0.18</td>
<td>0.82 ± 0.38</td>
<td>7.65 ± 0.4</td>
</tr>
<tr>
<td>Enrofloxain</td>
<td>10</td>
<td>5.72*** ± 0.14</td>
<td>6.84*** ± 0.11</td>
<td>9.09*** ± 0.2</td>
<td>33.12*** ± 0.17</td>
<td>0.57 ± 0.17</td>
<td>6.74 ± 0.16***</td>
</tr>
</tbody>
</table>

*** significant at p < 0.001
Table (3): Effect of enrofloxain on body weight of rats mean± S.E (n=10)

<table>
<thead>
<tr>
<th>G.</th>
<th>Dose/mg 100g.b.wt</th>
<th>Body weight in grams entry week</th>
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<tr>
<td></td>
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<td>1w</td>
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<tr>
<td>C</td>
<td>-</td>
<td>84 ± 2.64</td>
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<tr>
<td>Enrofloxain</td>
<td>7.5</td>
<td>88 ± 2.47</td>
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<td>80±1.47</td>
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* Sig.at p<0.05
*** Sig. at P<0.001

References


