ANETHUM GRAVEOLENS (DILL) MITIGATES THE LORNOXICAM INDUCED TOXICITY IN RATS

Ashraf Elkomy¹, Madline Afify¹, Faten El sayed¹, Ahmed Abdeen², Afaf Abdelkader³

¹Department of Pharmacology, Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt
²Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Benha University, Toukh 13736, Egypt
³Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Benha University, Benha 13518, Egypt

ABSTRACT

Lornoxicam (LOR) is a non-steroidal anti-inflammatory drug known to induce hepato-renal toxicity. The following study aimed to investigate the adverse effect of LOR in oxidative status beside biochemical and histological examination of Liver and kidney tissue as well as the protective effect of Dill. Thirty six male Wister albino rats were randomly divided into main four groups of nine rats each were used; G (1) Control group and they were administered 0.5ml of saline orally for 21 consecutive days, G (2): they were administered 2g/kg dill orally for 21 consecutive days, G (3): they were administered 1.3 mg/kg body weight/day of LOR intraperitoneally for 21 consecutive days, G (4): they were administered 2g/kg dill orally concurrently with 1.3 mg/kg body weight/day of LOR. The results revealed that LOR induced significant increases in liver and kidney function parameters including AST, ALT, creatinine, and urea. Disrupted lipid metabolism also was observed in LOR-treated animals. Moreover, marked increase in malondialdehyde (MDA) and decreases in glutathione (GSH) and catalase (CAT) following LOR-insult suggested a possible involvement of lipid peroxidation in LOR-induced hepatorenal and gastric toxicity. Oral administration of dill at the dose 2g/kg for 21 days was significantly mitigating these toxic effects. So it is concluded that dill has great antioxidant and antiapoptotic effect.

Keywords: Lornoxicam, Dill, hepato-renal toxicity, Oxidative Stress, lipid peroxidation

1. INTRODUCTION

Lornoxicam (LOR) is a new drug of oxicam category non-steroidal anti-inflammatory (NSAID), widely consumed as it has an analgesic, anti-inflammatory and antipyretic consequence and extensively prescribed in management of rheumatoid arthritis, osteoarthritis, acute sciatica and low back pain (Tayal, 2012; Pagar et al., 2018).

In spite of the salutary effects of LOR, adverse effects limit its use. Thus, LOR has many toxic effects on the GIT, liver, kidney, CVS and others (Atac et al., 2015). LOR is known to exert its influence via suppressing the synthesis of prostaglandin by inhibiting the enzyme cyclooxygenase (both COX 1 and 2, without precise selectivity) through its competition with arachidonic acid
(Berg et al., 1999). Moreover, aside from prostaglandin suppression, a growing body of evidences indicates that oxidative damage is embroiled in LOR-induced hepatorenal and gastroduodenal injuries such as excessive production of free radicals or antioxidant deficiency (Sen et al., 2006; Abdeen et al., 2019).

Moreover, reactive oxygen species (ROS) are cytotoxic molecules naturally produced in all cells during cellular respiration which rapidly balanced by the endogenous antioxidant defense mechanism which can result in protein oxidation, lipid peroxidation and DNA damage along with inflammatory initiation and intrinsic apoptotic pathways (Sen et al., 2006; Avery et al., 2011; Small et al., 2012). Glutathione (GSH), catalase (CAT), glutathione peroxidase (GSHPx), and superoxide dismutase (SOD) are major natural intracellular scavengers that take a crucial role in keeping the redox homeostasis, that way, protecting the cellular molecules from the cytotoxic impacts of surplus ROS (Abdeen et al., 2019). Anethum graveolens (Dill) is an aromatic medicinal herb and seasoning belonging to the Apiaceae family (Jana et al., 2012). Dill possesses antioxidants, anti-hyperlipidemia, anti-diabetic, anticonvulsant, anti-secretion activities as well as cardio protective and hepatoprotective properties (Mudassir et al., 2011; Oshaghi et al., 2017). Limonene, carvone and α-phellandrene are the major ingredient found in the dill extract which have robust antioxidant effect. Myristicin, p-cymene, anetophurane and dillapiole also forming bit components of dill (Carrubba et al., 2010). Corroborating evidence showed that, dill extracts and its ingredients effective in the treatment of acetaminophen (Ali 2013, Ramadan et al., 2013), gentamicin (Srivastava et al., 2018), diabetes (Goodarzi et al., 2016), CCl4 (Tamilarasi et al., 2012, Rabeh et al., 2014, Oshaghi et al., 2017), fungal infection (Tian et al., 2012), hepatocellular carcinoma (Mohammed et al., 2018) and hypercholesterolaemia (Hajhashemi and Abbasi, 2008).

Inasmuch as the ameliorative effect of dill against cellular damage and apoptosis caused by LOR in hepatic, renal and gastric tissues have not been investigated, the current research was destined to study the possible mechanisms of the anti-apoptotic and antioxidant activity of dill in opposition to acute LOR hepatorenal and gastric toxicity. Nephro-hepatic protections were determined by evaluation of serum liver and kidney function. Oxidative state in liver, kidney and stomach tissues was determined to confirm the protective influence of dill. A histopathological examination has been carried out to validate the preventive action. Immunostaining of caspase-3 in hepatic, renal and gastric tissues was performed to examine the anti-apoptotic success of dill.

2. MATERIALS AND METHODS

2.1. Drugs

Lornoxicam, LOR (8 mg vial) was purchased from Global pharmaceutical industries, Egypt. It was available in the form of lyophilized powder for IM or IV injection. Dill extract: Anethum graveolen seeds are ground and 100 g of powder was poured inside a percolator for 25 h with 300 mL of distilled water. Then the mixture was filtrated and condensed under pressure by spinning and desiccating. The aqueous extract yield (w / w) was 8.2% (g / g) (Monsefi et al., 2006).

2.2. Experimental study

The current study was conducted on a total number of 36 male Wister albino rats weighing 165-200 g. Rats were purchased from the Faculty of Veterinary Medicine, Laboratory Animal Center, Benha University, Egypt. For two weeks, they are acclimatized
before the experiment (temperature ~25 °C). They obtained a regular laboratory healthy commercial diet and water ad libitum. The Ethics Committee of the Faculty of Veterinary Medicine of the University of Benha approved the use of rats as well as the study protocol.

2.3. Experimental design

Rats are randomly designate to four equal groups (9 rats each). Group (1) (Control); rats used as the vehicle and be given 0.5ml of saline, i.p. Group (2) (dill); rats received dill orally once daily at a dosage of 2g / kg b.w. by stomach tube (Srivastava et al., 2018). Group (3) (LOR): rats were used as Lornoxicam toxic group and were injected 1.3 mg/kg b.wt. of Lornoxicam i.p. (Sen et al., 2006). Group (4) (LOR+dill): rats co-administered Lornoxicam (.3 mg/kgb.wt, i.p.) with oral dill (2g/kg b.wt.). Over 21 consecutive days, all medicines were given once daily.

2.4. Sampling of Blood and Tissue

At the end of the experimental study, rats were euthanized using sodium pentobarbital exposure and blood samples were obtained directly from medial eye canthus, part used for hematological tests and put in heparinized tubes and the other portion was used for biochemical analysis and stored at room temperature with no anticoagulant for effective serum separation.

Liver and kidney tissues were also harvested for oxidative cascade determination, and histopathological examinations.

2.5. Hematological and biochemical studies

To calculate the total number of erythrocytes and leukocytes, an automated cell counter (H.A-Vet Clindiage, Belgium) was used. The hemoglobin (Hb) concentration was determined. Serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are evaluated (Murray and Kaplan, 1984), total protein (Koller, 1984), albumin (Doumas et al., 1971), creatinine and urea (Murray and Kaplan, 1984). Furthermore, cholesterol and triglycerides were evaluated according to Ellefson and Garaway (1976) and Buccolo (1973), respectively. Test kits were obtained from the Diamond Test Laboratory (Egypt).

2.7. Histopathological examination

Tissue samples obtained in different groups from rats’ liver and kidneys and fixed for 24 hours to a 10% formalin solution. The obtained tissues were bathed under flowing water, dehydrated in an ascending sequence of ethanol, and then washed in xylene and wrapped in paraffin. Then we cut the paraffin block into 5-μm thick sections on glass slides and hematoxylin and eosin (H&E) stained for routine microscope inspection (Banchroft et al., 1996).

2.8. Immunohistochemical examination:

Sections of tissue were exposed to graded dilution of ethyl alcohol are deparaffinized and sequentially dehydrated.
Recovery of antigen was performed by heating the segments for 5 min in a solution of citric acid. The slide was incubated with anti-caspase-3 primary monoclonal antibody (Santa Cruz Biotechnology Inc., Dallas, TX, USA, 1:100 dilution) overnight after being blocked in 5% of BSA for 20 minutes. The slide was then rinsed 3 times with PBS followed by 37 °C incubation for 1 h to avidin-biotin complex of avidin-biotin complex (ABC pack, Vector Laboratories). The brown color was displayed with tetrahydrochloride (DAB) 3,3-diaminobenzidine (Dako, Japan). Then, the slide counterstained with Mayer's hematoxylin. All parts are imaged using a digital imaging light microscope (DM6 M LIBS, Leica Microsystems, Germany)

**Statistical analysis**

The findings are expressed as mean ± SE of the groups analyzed using variance analysis analyzes (one-way ANOVA) then by Duncan's multiple-range test to determine differences between the averages. All analysis was conducted by the Statistical System for Social Science Applications SPSS (16) Software (SPSS Inc., Chicago, USA).

3. **RESULTS**

3.1. **Hematological finding**

Table (1) shows a significant decrease in RBCs count and Hb levels but a dramatic increase in WBCs after LOR-intoxication compared to control rats. In comparison, dill therapy considerably enhanced RBC and Hb concentrations in LOR-treated rats to near-normal concentrations. In addition, dill considerably reduced the increase in WBCs in LOR-treated rats.

3.2. **Biochemical parameters**

As shown in Table (2), LOR therapy induced liver and kidney injury as elucidated by a dramatic increase in hepatic and renal biomarkers including AST, ALT, creatinine and urea concentrations. Such results are followed by substantial reductions in albumin, total protein, cholesterol and triglyceride levels in comparison to control animals. Alternatively, significant improvements in liver and kidney function and lipid profile were observed when LOR was concurrently administered with dill relative to LOR treated animals.

3.3. **Liver, kidney and stomach oxidative stress biomarkers**

The ameliorative impacts of dill against LOR-induced oxidative injury in hepatic, renal and gastric tissues are shown in Table (3). MDA levels have been dramatically improved together with significant decreases in GSH and CAT activity in all tissues in regard to LOR-intoxication suggesting oxidative damage. LOR-induced oxidative damage in gastrointestinal, liver and renal tissue were significantly improved by co-administration with dill. The values for the LOR+dill group were nearer to the control values.

3.4. **Histopathological examination**

In order to verify the findings described before, we investigated the histopathological changes that have occurred in the liver, kidney and stomach tissue after treating with LOR and/or dill. Histopathological changes in liver were shown in Fig. (1). In the control group, there was no histological alteration of the central vein and hepatocytes parenchyma was reported (Fig.1A). The same result was recoded in dill group (Fig.1B). While enormous inflammatory cell aggregation was identified in the portal area (Fig.1C) as well as dilation of the portal vein with freshly shaped bile ductules in the case of LOR treated group liver, (Fig.1D). The portal area exhibit periductal inflammatory cells infiltration (Fig.1E). Beneficially in the simultaneous group (Dill + LOR), the portal area showed limited infiltration of inflammatory cells (Fig.1F).
Histopathological changes in the kidney have been shown in Fig. 2. There were no alteration; normal glomeruli and cortical tubules were reported in the control (saline) group and in the dill group (Fig.2 A and B, respectively). Kidney of LOR treated group showed, severe cortical blood vessels dilatation (Fig.2C), while some of the tubules had eosinophilic casts formation in the lumen with focal fibrosis in between the tubules (Fig.2D). In concurrent group (Dill + lornoxicam), there was no histopathological alteration was recorded (Fig.2E).

As shown in Fig. 3, stomach sections of saline and dill treated rats (Fig.3 A, B and C, respectively) control group shows, normal histological mucosal surface structures with lamina propria and glandular structure as well as underlying submucosa muscularis and serosa, respectively. Fig. D, E: LOR-intoxicated rat exhibits significant injury of gastric surface epithelium, intracellular chief cells vacuolation, pyknosis of nuclei, inflammatory cell infiltration and fibrosis in the submucosal layer. F: control dill shows, no histological changes. G,H: LOR+dill group shows, enhancement of histological appearance of the stomach.

3.5. Immunohistochemical examination
Increases in activated caspase-3 expression following LOR and/or dill therapy in liver, kidney and stomach tissue are described Figs. 4–6. LOR evidently strengthened activated expression of caspase-3 in hepatic (Fig. 4B), renal (Fig. 5B) and gastric (Fig. 6B) cells showed activation of apoptotic pathways compared to the control group. Moreover, when rats were treated with dill, we notice moderate expression of activated caspase-3 (Figs. 4–6D) in all examined tissues confront to LOR- intoxicated rats.

The reaction (brown dark coloration) in liver is localized intracellular in hepatocytes while in kidney in lining tubular epithelium and in stomach at mucosal epithelium.

4. DISCUSSION
Amongst NSAIDs, LOR is prescribed widely due to its powerful action in controlling of pain and inflammation. Despite its long clinical success, growing evidences have shown that LOR has an adverse effect on the liver, kidneys and stomach (Atac et al., 2015).

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Lornoxicam</th>
<th>Dill</th>
<th>Lornoxicam +Dill</th>
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<tbody>
<tr>
<td>RBCs (10^12/L)</td>
<td>8.80 ± 0.18 a</td>
<td>6.67 ± 0.24 c</td>
<td>8.02 ± 0.06 b</td>
<td>7.60 ± 0.22 b</td>
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<td>WBCs (10^9/L)</td>
<td>8.87 ± 0.09 b</td>
<td>16.49 ± 0.17 a</td>
<td>9.11 ± 0.37 b</td>
<td>15.17 ± 0.36 a</td>
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<td>Hb (g/dl)</td>
<td>15.64 ± 0.22 a</td>
<td>10.40 ± 0.29 c</td>
<td>15.28 ± 0.21 a,b</td>
<td>14.74 ± 0.26 b</td>
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Effect of lornoxicam and/or dill treatment on hematological parameters in rats (n=9). Lornoxicam (1.3 mg/kg); Dill (2g/kg); RBCs, red blood cells; WBCs, white blood cells; Hb, hemoglobin. Data are presented as mean ± SE. The values with different superscript letters are statistically significant at P≤0.05 when compared to each other (in the same row).
Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Lornoxicam</th>
<th>Dill</th>
<th>Lornoxicam +Dill</th>
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<tr>
<td>AST (U/L)</td>
<td>78.52±2.77&lt;sup&gt;c&lt;/sup&gt;</td>
<td>153.33±6.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.19±2.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>119.19±6.72&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>ALT(U/L)</td>
<td>36.33 ± 2.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>79.89 ± 3.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.15 ± 1.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>45.33 ± 2.92&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>T.protein (g/dl)</td>
<td>7.32 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.83 ± 0.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.01 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.58 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Albumin (g/dl)</td>
<td>4.26 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.81 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.08 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.58 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Creatinine (mg/dl)</td>
<td>0.71 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.47 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.67 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.18 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Urea (mg/dl)</td>
<td>44.25±1.76&lt;sup&gt;c&lt;/sup&gt;</td>
<td>83.54±2.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.84±2.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50.45±3.20&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Cholesterol (mg/dl)</td>
<td>89.33±1.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.56±1.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.56±1.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69.11±2.09&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Triglyceride (mg/dl)</td>
<td>69.22±1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.11±2.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.22±1.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.67±2.15&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

Effect of lornoxicam and/or dill treatment on serum biochemical parameters in rats (n=9). Lornoxicam (1.3 mg/kg); Dill (2g/kg); AST, aspartate aminotransferase; ALT, alanine aminotransferase; T. protein, total protein. Data are presented as mean ± SE. The values with different superscript letters are statistically significant at $P\leq0.05$ when compared to each other (in the same row).

In addition to the non-selective anti-COX action of LOR, there is that oxidative damage and ROS generation, development of activation cytochrome p450, oxidative phosphorylation uncoupling, are involved in various tissue injuries (Chung et al., 2006).

When there is a mismatch between antioxidant potential and production of ROS, the cell is at risk of severe oxidative stress. Consequently, ROS can disrupt cell membranes and other cellular molecules; bring about unfolding of protein, DNA oxidation, lipid peroxidation, mitochondrial dysfunction, and ATP depletion leading to cell breakdown and death (Abdel-Daim et al., 2015).

Table 3

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<thead>
<tr>
<th>Parameter</th>
<th>control</th>
<th>Lornoxicam</th>
<th>Dill</th>
<th>Lornoxicam +Dill</th>
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<tr>
<td>Liver</td>
<td></td>
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<tr>
<td>MDA (nmol/gm)</td>
<td>273.84 ± 23.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>502.48 ± 43.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>271.48 ± 24.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>300.09 ± 26.48&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>GSH (mg/gm)</td>
<td>56.26 ± 7.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.90 ± 1.32&lt;sup&gt;d&lt;/sup&gt;</td>
<td>51.51 ± 1.66&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>46.57 ± 1.81&lt;sup&gt;b,c&lt;/sup&gt;</td>
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<td>CAT (U/gm)</td>
<td>485.28 ± 8.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>436.14 ± 14.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>482.48 ± 6.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>475.00 ± 12.44&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Kidney</td>
<td></td>
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<tr>
<td>MDA (nmol/gm)</td>
<td>57.13±4.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>179.18±15.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.47±4.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>118.43±9.69&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>GSH (mg/gm)</td>
<td>66.08 ± 2.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.17 ± 3.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.64 ± 2.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.52 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>CAT (U/gm)</td>
<td>508.35±7.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>437.30±18.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>508.09±8.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>480.83±2.96&lt;sup&gt;b&lt;/sup&gt;</td>
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Effect of lornoxicam and/or dill treatment on oxidative stress markers in liver, and kidney tissues in rats (n=9). Lornoxicam (1.3 mg/kg); Dill (2g/kg); MDA, malondialdehyde; GSH, reduced glutathione; CAT, catalase. Data are presented as mean ± SE. The values with different superscript letters are statistically significant at $P\leq0.05$ when compared to each other (in the same row).
Hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (OH$^-$), superoxide anion (O$_2$$^-$) and nitric oxide (NO) are the main ROS formed during oxidative stress that squelch the intracellular antioxidant system. Of them, OH$^-$ is considered to be the most harmful one that has direct insult on the cell membrane lipid causing lipid peroxidation, and causing enhanced levels of MDA. To make matters worse, the formed MDA fasten the other cellular molecules which exacerbate the situation. This finding elucidated that, cell membrane injury hepatic, renal and gastric cells is ascribed to the increase production of OH$^-$. Moreover, H$_2$O$_2$ hydrolyzed into H$_2$O by the antioxidant (mainly GSH, CAT, and SOD) however, consumption of such antioxidants shift to Fenton’s reaction, where generous quantity of OH$^-$ are created from H$_2$O$_2$ (Sies et al., 2017; Abdeen et al., 2019).

On the other hand, NO reacts with oxygen and leads to formation of oxidized form of nitrogen causing further cellular injuries (Oshaghi et al., 2017; Abdeen et al., 2019).

On the basis of these considerations, our study found that extreme hepatorenal toxicity following treatment with LOR suggested by substantial increases in serum AST and ALT production, creatinine and urea concentrations, together with a significant decrease in total serum protein and albumin compared to control. Such results complement the data obtained from previous reports by Aabakken et al., (1996), Misraulia (2002), Pehlivan et al., (2010), El-zoghby and Farok (2012), Salah-Eldin et al., (2012), Donati et al., (2016) and Bahr et al., (2016).
Figure (1): Histology of liver tissue after treatment with lornoxicam (LOR) and/or dill. A and B: liver sections from saline (control) and dill-only treated rats, respectively, show normal hepatic architectures. C, D, E: LOR-intoxicated rat shows, massive inflammatory cells aggregation in portal area, dilation of portal vein, periductal inflammatory cell infiltration surrounding the bile ducts, focal inflammatory cell infiltration in the parenchyma, respectively. F: LOR+dill group shows, few inflammatory cell infiltration, and apoptosis in some hepatocytes. H&E stained sections.
Figure (2) Histology of kidney tissue after treatment with lornoxicam (LOR) and/or dill A and B: Renal sections from saline (control) and dill-only treated rats, respectively, normal architecture of glomeruli and renal tubules at the cortex. C and D: LOR-intoxicated rat shows, severe dilation in cortical blood vessels, eosinophilic casts formation in the lumen with focal fibrosis in between the tubules, respectively. E: LOR+dill group shows, no histopathological alteration. H&E stained sections
Figure 3: Histology of gastric mucosa after treatment with lornoxicam (LOR) and/or dill. A, B, and C: control group shows, normal histological structure of the mucosal layer with lamina propria and glandular structure as well as underlying submucosa muscularis and serosa, respectively. D, E: LOR-intoxicated rat shows, severe loss of gastric lining epithelial cells, cytoplasmic vacuolation of chief cells, pyknotic nuclei, lymphocytic cell infiltration and fibrosis in submucosal layer. F: control dill shows, no histological changes. G,H: LOR+dill group shows, improvement in the gastric histological appearance. H&E stained sections
Figure 4: changes in liver caspase-3 expression after treatment with lornoxicam (LOR) and/or dill. A: control group. B: LOR-intoxicated rat. C: control dill. D: LOR+ dill group. Immunostaining was performed using anti-caspase-3 antibody and developed with DAB. The brown hepatic cell nuclei that are positively stained for activated caspase-3 are indicated by arrows. (magnification: X100).
**Figure 5:** changes in kidney caspase-3 expression after treatment with lornoxicam (LOR) and/or dill. **A:** control group. **B:** LOR-intoxicated rat. **C:** control dill. **D:** LOR+dill group. Immunostaining was performed using anticaspase-3 antibody and developed with DAB. The brown renal cell nuclei that are positively stained for activated caspase-3 are indicted by arrows (magnification: X100).
The elevated AST and ALT clarified by the increased permeability of the hepatocyte cell membrane as a consequence of lipid peroxidation in the hepatocyte leading to seepage of transaminases into the bloodstream (Hyder et al. 2013, Woreta and Alqahtani 2014, Abdeen et al., 2019).

Corroborating evidence attributed the LOR-induced remarkable reduction of total proteins and albumin concentrations are related to its toxic effects on hepatic cells causing its damage and immunosuppressive effect as plasma proteins primarily synthesized in the liver and a smaller percentage of lymphocytes and plasma cells are formed in the form of immunoglobulin (Chang et al., 1998, Kanwal et al., 2012). Also, gastrointestinal inflammatory lesions are condemned in protein loss. These results are similar to those reported in the literature of Atzpodien et al. (1997), Chang et al. (1998), (Misraulia, 2002) and (El-zoghby and Farok (2012).

In the current investigation, there was a marked decrease in RBC count and Hb concentrations in LOR-treated rats. Mounting evidence indicates that oxidative stress plays...
an important mechanism in the pathogenesis of hypochromic anemia caused by LOR by inhibition of both DNA and protein synthesis (Amare and Girmay, 2009). Besides, this could be explained by bleeding from ulceration of gastro duodenal mucosa induced by NSAIDs (Abdeen et al., 2019). These results complement the data obtained from previous reports by Misraulia (2002) and Abatan et al. (2006).

Also, our data elucidates a leukocytosis which might be due to excess inflammatory exudates in response to LOR insult. In conjunction with the present findings, those of El-Banhawy et al. (1994) and Mcafferty et al. (1995) who advocated that plenty of leucocytes were evident in response of body tissues confrontation of any injurious influence.

Our results divulge increased peroxidation of lipid synchronous with decreased levels of intrinsic antioxidants (SOD, GSH and CAT) with drastically increase in MDA, point out the involvement of oxidative distress and failure of antioxidant defense mechanisms to prevent the evolution of extravagant free radicals during LOR insult on liver and kidney. This result is consistent with that obtained by Sen et al. (2006).

Consistently with Le Bail et al. (1990), Hummdi et al. (2010), El-zoghy and Farok (2012), and Atac et al. (2015), our histopathological findings indicated liver injuries in the form of extensive inflammatory cells aggregation as well as dilatation of the portal vein. Also, hypertrophy of Kupffer's cells due to phagocytosis of cellular debris as defense activity. Also, histology of LOR-induced renal injury showed severe dilation in the blood vessels of the cortex, with focal fibrosis and inflammatory cells exudates in between the glomeruli and tubules. These results are in accordance with the previous research such as that reported by El-zoghy and Farok (2012).

Stomach of LOR intoxicated rat showed severe inflammation with extensive polymorphonuclear cells exudate, fibrosis with glandular architecture demolition and edema in submucosal layer. These findings, in the same vein with study done by Sen et al. (2006). This caused by suppression of prostaglandins which play a pivotal role in protection of gastroduodenal mucosal via inhibition of gastric acid secretion (Balfour et al., 1996, Parada et al., 2016).

Apoptosis is a physiological process for removing unwanted cells during development and for maintain tissue hemostasis (Mendez et al., 2014). Caspase are pivotal markers of apoptosis amongst them; Caspase-3 is considerably activated death protease, stimulate the specific cleavage of many crucial cellular protein and lead to DNA breakdown (Porter and Janick, 1999).

The current investigation elucidated, up regulation of the expression of caspase-3 in liver, kidney and stomach in LOR treated rats. On the basis of these results we suggest that apoptosis is embroiled in the pathogenesis of LOR-related toxicity.

Extract of dill leaves is deemed as phytochemical compounds contain generous amounts of phenolic and flavonoid such as Anthocyanins and Tannins (Agrawal et al., 2013). These components have an antioxidant property and capable to quill lipid peroxidation and then hepatorenal and gastric protection. Some properties of dill make it be eligible as antioxidant such as hydrogen donor, reducing metabolite, ROS scavenger, and chelating agent. Dill had a greater chelating potential against the ferrous which known as the strong prooxidant. Also, Dill is considered a strong NO scavenger (Oshaghi et al., 2017).
In line with this assertion, treatment with dill conferred marked amelioration against LOR tissues injury, as demonstrated in the current study, by worthy improvement of oxidative markers, histopathology and biochemical parameters but did not return to normal levels which confirm the protective role of dill against hepatic, renal and gastric toxicity induced by LOR. Fortunately, the present study corroborating that dill could ameliorate the effect of LOR on the expression level of caspase-3 in the concurrent group.

5. CONCLUSION:
Overall, these results indicate that mitochondrial dysfunction plays a pivotal role in LOR induce hepatotoxic, nephrotoxic and gastric effects notably ROS generation and lipid peroxidation, in conjunction with DNA damage, protein alteration and apoptotic cell death. It is pertinent to note that co-treatment with dill ameliorate LOR- induced lipid peroxidation in liver, kidney and gastric mucosa.

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