Side effects of dexibuprofen have been ameliorated with vitamin E
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A B S T R A C T

The present study was designed to define the effects of daily administration of 100 mg vitamin E/kg on side effects in mice treated with dexibuprofen (7.2 mg/kg, P.O once daily) for 21 consecutive days and its antioxidant scavenging capacity. Blood samples were collected from mice on 1st, 2nd, 14th, 21st days post –treatment and tissue samples were collected on 7th, 14th days post-treatment to assess the protective effects of vitamin E. The results indicated significant increase in antioxidant enzymes Glutathione peroxidase, dismutase superoxide dismutase (SOD) & catalase (CAT) together decrease the level of malondialdehyde besides diminished and in portal fibrosis and decrease in congestion of hepatic blood vessels and sinusoids caused by dexibuprofen administration as demonstrated by kidney histopathology Therefore, Vitamin E should be taken with dexibuprofen to decrease its side effects.

Key words Antioxidant activity, Dexibuprofen, Histopathology, Vitamin E.

1. INTRODUCTION

Dexibuprofen is non-steroidal anti-inflammatory drug. It's the active dextrorotatory enantiomer of ibuprofen. Most ibuprofen formulations contain a racemic mixture of both isomers (Hardikar, 2008). Dexibuprofen is the single pharmacologically effective enantiomer of Rac-ibuprofen. Rac-ibuprofen and dexibuprofen differ in their physic-chemical properties, in terms of their pharmacological properties and their metabolic Dexibuprofen has proven at least comparable efficacy to diclofenac, naproxen and celecoxib and has shown a favorable tolerability (Kaeheier et al., 2003). Antiplatelet effect of dexibuprofen (maximal inhibition of aggregation was 48-55% for adenosine diphosphate and 90-95% for collagen and arachidonic acid) was equal to the effect of aspirin The main difference between the two drugs was in the degree of recovery of platelet function. The effect of aspirin persisted for 24 h after the last dose (remaining inhibition 50% respect to the pretreatment value), whereas platelet aggregation had returned to baseline pretreatment values within 24 hr. after dexibuprofen was stopped (Gonzalez et al., 2007).

Dexibuprofen has an equal efficacy and comparable safety and tolerability with celecoxib in treatment of osteoarthritis. Dexibuprofen shows excellent tolerability, safety than other low-density lipoprotein like diclofenac sodium, dexibuprofen has stronger pain reducing effect than racemic ibuprofen (Kumaresan, 2010). Dexibuprofen-antioxidant conjugates were synthesized with the aim to reduce its
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gastrointestinal effects. The esters analogs of dexibuprofen were obtained by reacting its -COOH group with chloroacetyl derivatives. The in vitro hydrolysis data confirmed that synthesized prodrugs were stable in stomach while undergo significant hydrolysis in 80% human plasma and thus release free dexibuprofen. The minimum reversion was observed at pH 1.2 suggesting that prod rugs are less irritating to stomach than dexibuprofen. The anti-inflammatory activity is more significant than the parent dexibuprofen exhibited that synthesized prodrugs formed stable complexes with the COX-2 protein therefore concluded that the synthesized prodrugs have promising pharmacological activities with reduced gastrointestinal adverse effects than the parent drug (Ashraf et al., 2016).

Dexibuprofen is as effective and tolerable as ibuprofen. A dose of 5 mg kg⁻¹ and 7 mg kg⁻¹ dexibuprofen in place of 10 mg kg⁻¹ ibuprofen would be sufficient to control fever caused by URTI in children (Yoon et al., 2008).

The aryl propionic acid derivative dexibuprofen was the most potent antiplatelet drug, and its pharmacodynamics profile is similar to aspirin (De La Cruz et al., 2010). Non-steroidal anti-inflammatory drugs including selective cyclo-oxygenase-2 COX-2 inhibitors COXIBs are commonly used to treat acute gout. Published guidelines recommend their use to treat acute attacks, using maximum recommended doses for a short time (Van Durme et al., 2014).

In the lipid phase, tocopherols and carotenes as well as oxy-carotenoids are of interest, as are vitamin A and ubiquinols. In the aqueous phase, there are ascorbate, glutathione and other compounds imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage, is termed ‘oxidative stress’. Oxidants are formed as a normal product of aerobic metabolism but can be produced at elevated rates under pathophysiological conditions. Antioxidant defense involves several strategies, both enzymatic and non-enzymatic. In addition to the cytosol, the nuclear and mitochondrial matrices and extracellular fluids are protected. Overall, these low molecular mass antioxidant molecules add significantly to the defense provided by the enzymes superoxide dismutase, catalase and glutathione peroxidases (Sies., 1997). Defenses against free radical damage include tocopherol vitamin E, ascorbic acid vitamin C beta-carotene, glutathione, uric acid, bilirubin, and several metalloenzymes including glutathione peroxidase selenium), catalase, iron, and superoxide dismutase copper zinc manganese and proteins such as ceruloplasmin copper superoxide dismutase has powerful anti-inflammatory activity (Mcginness et al., 1978). For example, superoxide dismutase is a highly effective experimental treatment of chronic inflammation in colitis treatment with superoxide dismutase decreases reactive oxygen species generation and oxidative stress and thus inhibits endothelial activation. Therefore, such antioxidants may be important new therapies for the treatment of inflammatory bowel disease (Seguí et al., 2004).

Dexibuprofen was approved that it had nephrotoxicity-hepatotoxicity in rats. Therefore, this study aims to hepato-nephro-protective effects of vitamin E (100 mg/kg, P.O. once daily) against dexibuprofen side effects given for 21 days when vitamin E upon it co-administered with dexibuprofen.

2. MATERIALS AND METHODS

2.1 Drugs and chemicals
Dexibuprofen was obtained as SERACTIL® 500 mg, tablets that was supplied by Gebro pharmaceutical industry). Dexibuprofen was dissolved in normal saline. And given at a dosage of 7.2mg/kg according to Pagets and Barnes (1964) to convert the dose of human to rat. Vitamin E (vitamin E capsule) it was supplied by Pharco Pharmaceutical CO Alex., Egypt. Vitamin E dissolved in corn oil.

2.2 Animals
The present study was carried on 80 adult male albino rats weighing 150-200g. Animals were purchased from Laboratory
Animal Farm, Faculty of Veterinary Medicine, Zagazig University. All animals were housed in polypropylene cages with wood–chip bedding and were maintained on 12 hr./dark schedule in a temperature and humidity-controlled room. All animals were given access to food and water ad libitum. The animals were kept under observation for two weeks before using to ensure stabilization.

2.3 Experimental design
A parallel study design was adapted as 4 groups. The 1st group (control): rats in this group were not medicated and received normal saline, the 2nd group (Vitamin E) rats in this group received repeated oral doses of vitamin E (100 mg/kg B. WT.) for successive 21 days as an antioxidant, once daily. Rats were classified into 4 groups each contain 20 rats. The 3rd group (Dexibuprofen therapeutic dose) rats in this group received repeated oral doses of dexibuprofen (7.2 mg/kg b. wt.) for successive twenty two days, once daily. The 4th group (Dexibuprofen and Vitamin E (Dex. + Vit. E)) rats in this group received repeated oral doses of dexibuprofen (7.2 mg/kg b. wt.) for successive 21 days plus vitamin E (100 mg/kg B. WT.) once daily for 21 days.

2.4 Preparation of serum and tissue sample
At the end of experiment (24 hrs. after the last dose) rats were scarified and blood samples collected in a sterile Wasserman tube without anticoagulant from 5 rats/group at the 1st, 7th, 14th, 21st days post treatment and allowed to clot for 30 minutes and serum was separated by centrifugation at 3000 rpm for 15 minutes, and the top yellow layers of serum were pipette off without distributing the white buffy layer. Serum was stored at 20°C in Eppendorf tubes till the time of the work for determination of serum level of antioxidant enzymes. The liver and the kidney of each rats were collected at 7th and 14th days post-treatment. They were isolated and kept in 10% phosphate–buffered formalin for histopathological evaluation.

2.5 Biochemical markers of antioxidant activity

Determination of malondialdehyde (MDA), glutathione peroxidase activity (GPX), superoxide dismutase activity, catalase activity (CAT) according to the method described formerly (Aebi, 1984; Nishikimi et al., 1972; Paglia and Valentine, 1967, respectively).

2.6 Renal and hepatic histopathological evaluation
Kidney and liver tissues were fixed in 10% neutral buffered formalin solutions for 24 hours. Then tissue processing and paraffin blocks preparation were done. Masson, trichrome and hematoxylin-eosin stains were used to evaluate fibric areas and necro inflammation activity (Suvarna et al., 2013).

2.7 Statistical analysis
Statistical analyses were carried out by the one-way analysis of variance (ANOVA) (Tamhane and Dunlop, 2000).

3. RESULTS

3.1. Effects of dexibuprofen, Vitamin E and their combination on antioxidant enzymes.
Effect of combination between Dexibuprofen and Vitamin E on Determination of MDA level in the 1st day, there was a decrease in amount of MDA (15.426 ± 0.958 nmol/ml) compared with (20.56 ± 0.994 nmol/ml) for dexibuprofen group. On the 7th day resulted on a decrease in the amount of MDA (10.14 ± 0.123 nmol/ml) compared with (14.01 ± 0.751 nmol/ml) for the dexibuprofen group. On the 14th day resulted in a decrease in the level of MDA (8.366 ± 0.389 nmol/ml) compared with (10.14 ± 0.262 nmol/ml) for the dexibuprofen group. On the 21st day, there was decrease in the level of MDA (7.47 ± 0.068 nmol/ml) compared with (8.10 ± 0.184 nmol/ml) for dexibuprofen group as shown in table (1).

Effect of combination between Dexibuprofen and Vitamin E on GPX. On the 1st day resulted in an increase in GPX activity (91.59 ± 1.37 U/L) compared with (78.45 ±
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5.02 U/L) for dexibuprofen group. On the 7th day there is an increase in GPX activity (100.30 ± 3.17 U/L) for dexibuprofen group. On the 14th day there is an increase in GPX activity (110.11 ± 0.25 U/L) for the dexibuprofen group.

Effect of combination between Dexibuprofen and Vitamin E on superoxide dismutase. In the 1st day resulted in a highly significant increase in superoxide dismutase activity (9.33 ± 0.674 U/ml) compared with (6.39 ± 0.472 U/ml) for dexibuprofen group. In the 7th day resulted in an increase in superoxide dismutase activity (15.00 ± 0.663 U/ml) compared with (9.92 ± 0.569 U/ml) for dexibuprofen group. In the 14th day resulted in an increase in superoxide dismutase activity (18.26 ± 0.524 U/ml) compared with (15.84 ± 0.776 U/ml) for dexibuprofen group. In the 21st day resulted in an increase in superoxide dismutase activity (21.23 ± 0.484 U/ml) compared with (19.16 ± 0.626 U/ml) for dexibuprofen group.

Effect of combination between Dexibuprofen and Vitamin E on CAT. In the 1st day there was an increase catalase activity (191.85 ± 1.64 U/L) compared with (175.55 ± 5.12 U/L) for dexibuprofen group. In the 7th day resulted in an increase in catalase activity (204.06 ± 4.08 U/L) compared with (186.23 ± 4.17 U/L) for dexibuprofen group. In the 14th day resulted in an increase in catalase activity (214.07 ± 3.04 U/L) compared with (197.26 ± 4.44 U/L) for dexibuprofen group. In the 21st day resulted in an increase in catalase activity (227.39 ± 2.52 U/L) compared with (205.59 ± 3.10 U/L) for dexibuprofen group.

Table (1): The effect of Vitamin E (100 mg/kg, P.O. once daily), dexibuprofen (7.2 mg/kg, P.O. once daily), and their combination for 21 consecutive days on serum antioxidant markers activities of rats at 1st, 7th, 14th and 21st days of drugs withdrawal

<table>
<thead>
<tr>
<th>Time</th>
<th>Groups</th>
<th>CAT (µ/L)</th>
<th>SOD (µ/ml)</th>
<th>GPX (µ/L)</th>
<th>MDA (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>241.39±3.49</td>
<td>22.54±1.69</td>
<td>112.93±1.92</td>
<td>7.04±0.141</td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>247.28±5.12</td>
<td>23.90±2.05</td>
<td>111.40±3.30</td>
<td>6.75±0.506</td>
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<tr>
<td></td>
<td>Dexibuprofen</td>
<td>175.55±2.96</td>
<td>6.39±0.472</td>
<td>78.45±5.02</td>
<td>20.56±0.994</td>
</tr>
<tr>
<td></td>
<td>Dex. + Vit. E:</td>
<td>191.85±1.64</td>
<td>9.33±0.674</td>
<td>91.59±1.37</td>
<td>15.42±0.958</td>
</tr>
<tr>
<td>1st Day</td>
<td>Control</td>
<td>233.77±2.47</td>
<td>20.91±1.19</td>
<td>116.04±3.99</td>
<td>7.11±0.522</td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>235.01±4.91</td>
<td>22.58±0.854</td>
<td>113.51±1.457</td>
<td>7.52±0.343</td>
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<tr>
<td></td>
<td>Dexibuprofen</td>
<td>186.23±4.17</td>
<td>9.92±0.569</td>
<td>87.80±3.37</td>
<td>14.01±0.751</td>
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<td></td>
<td>Dex. + Vit. E:</td>
<td>204.06±4.08</td>
<td>15.00±0.663</td>
<td>103.76±2.97</td>
<td>10.14±0.123</td>
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<tr>
<td>7th Day</td>
<td>Control</td>
<td>228.78±8.22</td>
<td>20.04±0.626</td>
<td>116.04±3.99</td>
<td>7.05±0.533</td>
</tr>
<tr>
<td></td>
<td>Vitamin E</td>
<td>224.14±8.02</td>
<td>21.59±0.994</td>
<td>111.25±5.83</td>
<td>7.64±0.142</td>
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<tr>
<td></td>
<td>Dexibuprofen</td>
<td>197.26±4.44</td>
<td>15.84±0.776</td>
<td>100.30±3.17</td>
<td>10.49±0.262</td>
</tr>
<tr>
<td></td>
<td>Dex. + Vit. E:</td>
<td>214.07±3.04</td>
<td>18.26±0.524</td>
<td>110.07±0.587</td>
<td>8.36±0.389</td>
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<tr>
<td>14th Day</td>
<td>Control</td>
<td>229.669±5.25</td>
<td>21.58±0.928</td>
<td>118.48±2.75</td>
<td>7.43±0.076</td>
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<tr>
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<td>Vitamin E</td>
<td>227.58±5.06</td>
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<td>114.60±4.82</td>
<td>7.66±0.163</td>
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<tr>
<td></td>
<td>Dexibuprofen</td>
<td>205.59±3.10</td>
<td>19.16±0.626</td>
<td>110.11±3.25</td>
<td>8.10±0.184</td>
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<tr>
<td></td>
<td>Dex. + Vit. E:</td>
<td>227.39±2.52</td>
<td>21.23±0.484</td>
<td>117.59±2.09</td>
<td>7.47±0.068</td>
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</tbody>
</table>

Dex. + Vit. E: Dexibuprofen plus Vitamin E. (n = 5, mean ± SE). Means with different superscripts within the same column per one time point were significantly different at P< 0.05.

3.2. Histopathological Changes
3.2.1. Dexibuprofen 7th day
Liver section revealed moderate congestion of hepatic blood vessels and sinusoids, portal
(mild) biliary proliferation and round cells aggregation, focal disorganization of some hepatocytes with degenerative changes and early necrotic changes. In some sections, the portal triads showed moderate aggregation of round cells and eosinophil's. The same cells were also interstitially aggregated. Portal edema was also seen (Figure 1).

The renal blood vessels were moderately congested with a wide spread perivascular edema. The renal pelvis sections revealed congested blood vessels, edema, degenerative changes in the surrounding tubules and focal sloughing of the epithelium. Characteristic multifocal myxoid changes in the renal stroma at different location with presence of bluish myxoid secretory materials were seen. The glomeruli were sporadically shrinked or lobulated, sometimes with massive periglomerular round cells aggregates and epithelial crescent formation. Multifocal interstitial round cells aggregation were seen. Most of the renal tubules showed degenerative changes mainly hydropic, sometime with early necrotic changes. Large number of the tubules in both cortex and medulla were cystically dilated sometime with hyaline casts inside some of them (Figure 2).

3.2.2. Dexibuprofen 14th day
3.2.2.1. Liver: Section showed moderate congestion of hepatic blood vessels, portal (mild) biliary proliferation with fibroplasia and round cells infiltration, focal interstitial aggregation of round cells and focal centrlobular and periporal degenerative changes of the hepatocytes mainly hydropic degeneration and fatty change beside some apoptotic changes in a few hepatocytes.

3.2.2.2. Kidney: Sections showed characteristic congestion of the renal blood vessels and perivascular edema. Some of the renal tubules were degenerated (cloudy swelling and hydropic) with early necrotic changes. Moderate number of the renal tubules particularly in the medulla were cystically dilated. A few number of the glomeruli were shrinked or lobulated in addition to periglomerular round cells infiltration (Figure 3).

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Figure (1): Photomicrograph of Liver showing moderate congestion of hepatic blood vessels (stars), portal mild biliary proliferation (arrow) and round cells aggregation (curved arrow), focal disorganization of some hepatocytes (arrowhead). In some cases, the portal triads showing moderate aggregation of round cells (curved arrow) and eosinophil's (closed arrow). H & E X 100 (A, C ), 400 (B, D)
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3.2.3. Dexi-ibuprofen + Vitamin E 7th day
3.2.3.1. Liver: Sections showed normal hepatic parenchyma with residual portal fibrosis and biliary hyperplasia. Minute interstitial leucocytic aggregation and mild focal centrilobular degenerative changes mainly hydropic degeneration and fatty change were seen. The Kupffer cells were prominently enlarged. The hepatic blood vessels and sinusoids were mildly congested (Figure 4).

3.2.3.2. Kidney: Sections revealed apparently normal nephron units in most parts of the kidney with preserved renal papillae and renal pelvis, the latter in some cases revealed mildly edematous stroma with healthy normal lining epithelium. Some of the renal tubules in both cortex and medulla were cystically dilated.

3.2.4. Dexibuprofen + Vitamin E 14th day
3.2.4.1. Liver: Sections from liver showed apparently normal hepatic parenchyma with residual fibrosis in the portal area, mild biliary proliferation and scanty minute interstitial leucocytic aggregations. The fibrotic areas and the previously degenerated or necrotic hepatocytes were ameliorated and appeared partially surrounded by regenerating hepatocytes.

3.2.4.2. Kidney: Sections showed apparently normal nephron units, renal papillae and renal pelvis. Some of the cortical tubules were mildly dilated.
4. DISCUSSION

Imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to
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damage ,is termed (oxidative stress) (Sies, 1997). The oxidative stress is thought to contribute to the initiation and progression of atherosclerosis. Free radicals catalyze lipid peroxidation so can cause damage to cellular and intracellular structures, so administration of anti-oxidants prevent oxidation of fatty acid in the cell (Droke and Loerch, 1989). Vitamin E act as a peroxyl radical scavenger, disabling the production of damaging free radicals in tissues, by reacting with them to form a tocopherol radical, which will then be reduced by a hydrogen donor (such as vitamin C) and thus return to its reduced state (Traber et al., 2011) Vitamin E is thought to have a role in preventing atherosclerosis by inhibiting the oxidation of low–density lipoprotein (Fuller et al., 1998).

Vitamin E is a potent naturally occurring lipid soluble antioxidant possesses the ability to directly quench free radicals and function as a membrane stabilizer. It protects critical cellular structures against the damage from oxygen free radicals and reactive products of lipid peroxidation (Goldfarb et al., 1996). Superoxide dismutase as an enzyme that alternately catalyzes the dismutation of the superoxide radical into either ordinary molecular oxygen or hydrogen peroxide was defined by (Hayyan et al., 2016). Catalase is a common enzyme found in nearly all living organisms exposed to oxygen (such as bacteria, plants, and animals). It catalyzes the decomposition of hydrogen peroxide to water and oxygen (Cheilikani et al., 2004). Vitamin E can protect against acute Liver necrosis induced by carbon tetrachloride (Maurizio et al., 1992). Vitamin E is known to act by breaking the antioxidant chain that prevent ROS–produced cell membrane damage (Factor et al., 2000).

In the present study it was observed that vitamin E administration to rats produced an appreciable improvement in the histopathological changes that occur in kidney and liver which associated with administration of Dexibuprofen, also Vitamin E normalized levels of MDA, catalase, glutathione peroxidase and superoxide dismutase The possible pathway can be explained through structure of vitamin E. The group of eight compounds that include four tocopherols and four tocotrienols. All eight feature achromane double ring, with a hydroxyl group that can donate a hydrogen atom to reduce free radicals, and a hydrophobic side chain which allow penetration into biological membranes. The antioxidant activity of vitamin E during the peroxidation of unsaturated lipids has been reviewed based on its reaction products. Vitamin E used in a dose (100 mg/kg, P.O. once daily) for 21 days to clarify the hepatic nephroprotective effects on rats. We found a significant changes in antioxidants markers (SOD, CAT, GPX and MDA levels). Histopathological results in liver at 7th day for dexibuprofen showed moderate congestion of hepatic blood vessels and sinusoids, mild biliary proliferation and round cells aggregation. On the 14th day showed moderate congestion of hepatic blood vessels, mild biliary proliferation with fibroplasias and round cells infiltration. On 7th day for Dexibuprofen + Vitamin E showed normal hepatic parenchyma with residual portal fibrosis and biliary hyperplasia Kidney for Dexibuprofen only on 7th day showed the renal pelvis revealed congested blood vessels, edema, degenerative changes in the surrounding tubules and focal sloughing of the epithelium. On 14th day showed characteristic congestion of the renal blood vessels and perivascular edema. In the 7th day for Dexibuprofen + Vitamin E the kidney sections showed revealed apparently normal nephron units in most parts of kidney with preserved renal papillae and renal pelvis. On the 14th day kidney sections showed apparently normal nephron units, renal papillae and renal pelvis. Vitamin E is well accepted as nature’s most effective lipid–soluble, chain-breaking antioxidant, protecting cell membrane from peroxidative damage (Kaehler et al., 2003). Antioxidant Vitamin E retard hepatic fibrosis in biliary obstructed rats (Parola et al., 1992). Vitamin E is known to act by breaking the antioxidant chain that prevent ROS-produced cell membrane. It decreases lipid peroxidation and protects against liver injury. It decrease liver fibrosis, tumor necrosis
factors, inflammation and hepatic porphyrin (Bradford et al., 2003). The increased level of antioxidants enzymes CAT, GPX, and superoxide dismutase resulted from administration of vitamin E might normalized the lipid peroxidation reaction and related biochemical changes which in turn protects the cells from the increase risk of peroxidative damage as result of administration of cytotoxic drugs was reported by (Beytut et al., 2003).

Dexibuprofen can produce several adverse effects as oxidative damage, it produce a significant lipid peroxidation (Khan et al., 2014). Oxygen free radicals probably derived via action of xanthine oxidase, the decrease in super oxide dismutase activity and depletion of mucosal glutathione contribute to the pathogenesis of dexibuprofen induced ulceration (Burak Cimen et al., 2003). Oxygen free radicals probably derived via the action of xanthine oxidase during use of non-steroidal anti-inflammatory drug that lead to decrease in superoxide dismutase activity and depletion of mucosal glutathione (Ivillegas et al., 2000).

5. CONCULIONS

It could be concluded that Dexibuprofen has hepatic and renal disturbance in rats; Vit. E has a protective effect against renal and hepatic disturbance, which may attribute to decrease the harmful effects of Dexibuprofen by inhibiting free radical formation and by restoration of the antioxidant systems. The combination of vitamin E and Dexibuprofen showed better results than Dexibuprofen alone.

6. REFERANCES

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