



Protective Effects of Vitamin E on Pantoprazole Adverse Effects

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ABSTRACT

This study was conducted to evaluate the possible protective effect of vitamin E (100 mg/kg) on Pantoprazole adverse effects (3.6 mg/kg) based on its antioxidant scavenging capacity and histopathological examination. Eighty rats were used as 4 separate groups, 20 rats per each. Group (I) is a control group received saline; Group (II) is a vitamin E group received Vit. E (100mg/kg BW,P.O once daily) for 21 successive days, Group (III) is a pantoprazole group received pantoprazole (3.6 mg/kg BW,P.O once daily) for 21 successive days, Group (IV) is pantoprazole and Vit. E group received pantoprazole and Vit. E once daily for 21 successive days. The adverse effects of pantoprazole and the protective effects of vitamin E were assessed in blood at day one, day 7, day 14 and day 21 after drug withdrawal and in tissue samples at 7th and 14th days after drug withdrawal. Our results show a significant increase in serum antioxidant enzymes (CAT- SOD- GPX) and a significant reduction in lipid peroxidation (MDA) in vitamin E treated group, In addition to show reduction in liver fatty changes with decrease in degenerative changes in hepatocytes induced after giving pantoprazole. Also, degenerative changes in renal tubular epithelium caused after giving pantoprazole were minimized by vit. E. For these reasons Vitamin E should be advised to be used concurrently with Pantoprazole to reduce its adverse effects.

Keywords: *Pantoprazole, Vitamin E, Histopathology, Antioxidant activity.*

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1. INTRODUCTION

Pantoprazole is one of proton pump inhibitors (PPIs) which is a derivative of benzimidazole. Pantoprazole is a pro-drug that must be activated in an acidic environment at lower PH after administration to be in active form, it diffuses into the gastric parietal cells through the basolateral membrane and bind to the proton pump (H⁺, K⁺ adenosine triphosphatase) to make the pharmacodynamic effects (Parson, 1996).

PPIs have proven to be highly efficacious, and they have a very effective therapeutic

action to reduce acid production by 90% to 95% in 24 hours, meaning that it is 10 to 100 times more effective than H₂ blockers (Sobrevia Elfau et al., 2010).

Pantoprazole used mainly for treatment of different related acid peptic disorders, including gastroesophageal Reflux disease (GERD), dyspepsia, peptic ulcer disease, gastropathy that may cause after NSAIDs administration and used with antibiotics in *Helicobacter pylori* eradication (Jungniekel, 2000).

Pantoprazole is acid unstable and is thus prepared as an enteric-coated tablet, which should not be crushed. Its absorption is rapid and reach to maximum concentration after 2.5 hours after giving the drug either in single or multiple oral 40mg doses. The absorption of pantoprazole is well metabolized through first-pass metabolism, its bioavailability is 77%. The drug may be taken regardless food or antacids and its apparent volume of distribution (V_d) is approximately 11.0 to 23.6 L, and the protein binding in serum is ~98%. Pantoprazole is mainly metabolized in the liver through the cytochrome P-450 system (Meyer, 1996).

Pantoprazole causes many side effects, the Food and Drug Agency of the United States listed many warnings about the possibility of hypomagnesaemia, increased risk of fractures which may occur, and the decrease in clopidogrel efficacy when co-administered with any of proton pump inhibitors (Asim and Abbas 2016). Pantoprazole usage also may increase risk of exposure to *Clostridium difficile*, osteoporosis, the risk of fractures, pneumonia, thrombocytopenia, rhabdomyolysis, anemia, iron deficiency, hypomagnesaemia, vitamin B12 deficiency and nephritis. (Wilhelm et al., 2013). Many studies reported the possible adverse effects of pantoprazole like acute interstitial nephritis, which developed after 6 weeks of treatment with pantoprazole (Klassen et al., 2013). Severe acute hepatitis may be also induced by pantoprazole (Cordes et al., 2003).

Antioxidants have been defined as substance which prevent the genesis of reactive oxygen species (ROS) or other oxidants, scavenge them or repair its induced damage (Koshter et al., 1995). Antioxidant defense system act as a stable and

symmetrical framework and each depends on the activity of the other. In healthy conditions, the stability lies somewhat in support of the reactive species so that they can accomplish their biological roles, so protect against damage occurs even in healing individual (Halliwell 1995). Antioxidants mission is to act by coordination and balancing system to protect tissues from free radicals produced (Evans and Halliwell 2001). Vitamin E (Vit. E) considered to be an important antioxidant in the biological system that decrease the peroxidation of un-structural lipids by a chain breaking free radical (FR), so it gives a share in the stability of cellular membranes (Koshter et al., 1995). Vitamin E is the most important lipid phase antioxidant (Esterbauer et al., 1991).

This research aims for evaluating the hepatonephro protective effects of vitamin E (100mg/kg) against pantoprazole (3.6mg/kg) side effects for three weeks when it is co-administered with pantoprazole. Through measuring of oxidative stress biomarkers. In addition to liver and kidney histopathological findings.

2. Materials and methods

2.1. Drugs and Chemicals:

Pantoprazole 40mg (PROTOFIX® 40mg) was supplied by Ramida Pharmaceutical Industries. Pantoprazole was prepared to be effective in an acidic medium by adding sodium bicarbonate as described by Detinger et al (2002). The dose was (3.6 mg/kg) after converting the human dose (40mg) to rat dose according to Paget and Barners (1964).

Vitamin E (Vit. E® 1000 mg) It was supplied by Pharco Pharmaceutical CO., Alex, Egypt. Vitamin E was diluted in corn oil, the dose was (100mg/kg) as described by Campo et al (2001).

2.2. Animals:

Eighty adult male albino rats their weights ranged from 150 to 200gm were used in the study. They were purchased from Animal Farm, Faculty of Veterinary Medicine, Zagazig university. They were kept in polypropylene cages with wood-chip bedding. All animals were given access to food and water *ad lib*. They were kept in a period of two weeks before being used to ensure stabilization.

2.3. Experimental design:

Rats were classified into 4 groups, each containing 20 rats. The 1st group (control) rats in this group are not medicated and received saline. The 2nd group (Vit. E) rats in this group received oral dose of vitamin E (100mg/kg BW) once daily for 21 successive days as standard antioxidant. The 3rd group (pantoprazole) rats in this group received oral dose of pantoprazole (3.6 mg/kg) for 21 successive days, once daily. The 4th group (pantoprazole and vitamin E) rats in this group received oral dose of pantoprazole (3.6 mg/kg) and vitamin E (100mg/kg) once daily for 21 days.

2.4. Preparation of serum sample and tissue sample:

At the end of the trial, rats were sacrificed, and blood samples were collected into a sterile Wasserman tubes without anticoagulant from 5 rats/group on the 1st, 7th, 14th, 21st days post treatment. Serum was separated through centrifugation at 3000 rpm for 15 minutes. Serum was stored at -20°C in Eppendorf tubes till the time of the work for determination of antioxidant enzymes serum level. The liver and the kidney of each rat were collected on 7th and 14th days post-treatment. They were removed and kept in 10% formalin for histopathological evaluation.

2.5. Biochemical markers of antioxidant activity:

Determination of catalase activity (CAT) was done according to Aebi (1984), superoxide dismutase activity (SOD), glutathione peroxidase activity (GPx), Nishikimi et al (1972) and malondialdehyde activity (MDA) as described by Palgia and Valentine (1967).

2.6. Hepatic and Renal histopathological evaluation:

Histopathology of liver and kidney was conducted according to Suvarna et al (2013).

2.7. Statistical analysis:

Data in the present study were statistically analyzed using prism version 6. Statistical evaluations of the results, except those histopathology scoring, were carried out by means of the one way and two-way analysis of variance (ANOVA) followed by Duncan's test $p < 0.05$ was considered statistically significant (Tamhane et al., 2000).

3. RESULTS**3.1. Antioxidant effect of pantoprazole (3.6mg/kg), Vitamin E (100mg/kg) and their combination for 21 consecutive days:****Impact of combination between pantoprazole and vit. E on CAT**

On the 1st day there was an increase in catalase activity (207.03 ± 8.77 U/L) compared with (197.41 ± 6.25 U/L) for the pantoprazole group. On the 7th day, a significant increase in catalase activity was observed (226.48 ± 13.01 U/L) compared with (206.45 ± 4.29 U/L) for the pantoprazole group. On the 14th day resulted in an increase in catalase activity (236.02 ± 9.65 U/L) compared with (220.98 ± 1.20 U/L) for the pantoprazole group. On the 21st day resulted in an increase in catalase activity ($251.94 \pm 6.$ /L) compare with (238.23 ± 1.57 U/L) for pantoprazole group, as shown in table (1).

Impact of combination between pantoprazole and vit. E on SOD

On the 1st day resulted in a highly significant increase in SOD activity (16.62 ± 0.66 U/ml) compared with (9.98 ± 1.09 U/ml) for pantoprazole group. On the 7th day resulted in an increase in SOD activity (20.41 ± 1.30 U/ml) compared with (16.48 ± 2.05 U/ml) for pantoprazole group. On the 14th day resulted in an increase in SOD activity (21.40 ± 1.24 U/ml) compared with (19.67 ± 1.66 U/ml) for pantoprazole group. On the 21st day resulted in an increase in SOD activity (23.48 ± 0.26 U/ml) compared with (21.47 ± 0.97 U/ml) for pantoprazole group, as shown in table (1).

Impact of combination between pantoprazole and Vit. E on GPx

On the 1st day, there was an increase in GPx activity (96.01 ± 2.94 U/L) compared with (90.58 ± 2.8 U/L) for Pantoprazole group, On the 7th day there is an increase in GPx activity (103.13 ± 5.73 U/L) compared with (97.28 ± 2.94 U/L) for pantoprazole group, On the 14th day there is an increase in GPx activity (110.54 ± 4.24 U/L) compared with (104.34 ± 0.38 U/L) for pantoprazole group, On the 21st day there is an increase in GPx activity (118.11 ± 4.73 U/L) compared with (118.04 ± 0.91 U/L) for the pantoprazole group, as shown in table (1).

Regarding the combination of pantoprazole and vit. E on MDA,

On the 1st day resulted in a significant decrease in MDA activity (18.15 ± 4.74 nmol/ml) compared with (24.91 ± 5.17 nmol/ml) for pantoprazole group. On the 7th day resulted in a decrease in MDA activity (10.85 ± 1.33 nmol/ml) compared with (16.96 ± 4.14 nmol/ml) for pantoprazole group. On the 14th day resulted in a decrease in MDA activity

(8.85 ± 1.54 nmol/ml) compared with (12 ± 1.58 nmol/ml) for pantoprazole group. On the 21st day resulted in decrease in MDA activity (7.21 ± 0.73 nmol/ml) compared with (8.33 ± 0.99 nmol/ml) for pantoprazole group, as shown in table (1).

3.2. Histopathological changes in 7th day post treatment

3.2.1. Pantoprazole 7th day post treatment: (3rd group)

Liver section revealed mild congestion of the portal blood vessels and biliary hyperplasia. Some of the hepatocytes (25-30%) showed cloudy swelling and the kuffer cells were hypertrophied.

Kidney section showed degenerative changes in the cortical renal tubular epithelium mostly cloudy swelling and hydropic degeneration. Some of the collecting tubules were dilated with partially atrophied lining epithelium. The intertubular capillaries were mildly congested (Fig.1).

3.2.2. Pantoprazole 14th day Post treatment: (3rd group)

Liver section revealed characteristic biliary hyperplasia with periductal fibroblast and extension of the proliferative reaction to connect adjacent portal triads. Moderate portal and interstitial round cells aggregations with presence of some apoptotic hepatocytes in between. Most of the hepatocytes showed cloudy swelling with obstruction of bile canaliculi. The portal blood vessels were mildly congested and the kuffer cells were hypertrophied.

Kidney section showed Mild perivascular edema and degenerative changes in the cortical and medullary tubular epithelium were seen. The tubules were dilated with partial atrophy of the lining epithelium. The

cortical and medullary blood vessels and intertubular capillaries were mildly congested. (Fig.2).

3.2.3. Pantoprazole + Vit. E 7th day post treatment:(4th group)

Liver section revealed apparently normal hepatocytes, however, few sections showed fatty changes in 25-30% of the hepatic cells. The portal area showed mild biliary proliferation and the kupffer cells were mildly hypertrophied.

Kidney section showed mildly congested with perivascular edema. The glomeruli were normal in most sections, however a few of them were mildly shrunk or lobulated. Degenerative and necrotic changes were seen

in some of the proximal convoluting tubules and some of the medullary collecting tubules with cystic dilation in a few of them. (Fig.3).

3.2.4. Pantoprazole+ Vit. E 14th day Post treatment:(4th group)

Liver section revealed normal hepatic parenchyma, portal structures and vascular tributaries, however a few hepatocytes showed cloudy swelling and the kupffer cells were mildly hypertrophied.

Kidney section showed mild congestion of renal blood vessels and dilated medullary collecting tubules with partial atrophy of the lining epithelium were seen in some sections. No abnormalities could be detected in other examined sections. (Fig.4).

Table1. The effect of Vit. E (100 mg/kg, P.O. once daily), Pantoprazole (3.6mg/kg, P.O. once daily), and their combination for 21 consecutive days on oxidative stress markers of rats on 1st, 7th, 14th and 21st days of drugs withdrawal (n=5, mean \pm SE).

Days	Groups	CAT (U/L)	SOD (U/ml)	GPX (U/L)	MDA (nmol/ml)
1 st Day	Control	242.86 \pm 1.30 ^a	24.63 \pm 2.43 ^a	114.46 \pm 2.41 ^a	6.41 \pm 0.22 ^b
	Vitamin E	242.48 \pm 0.65 ^a	24.99 \pm 1.87 ^a	115.60 \pm 1.54 ^a	6.28 \pm 0.44 ^b
	Pantoprazole	197.41 \pm 6.25 ^b	9.98 \pm 1.09 ^c	90.58 \pm 2.80 ^b	24.91 \pm 5.17 ^c
	Pantoprazole + Vit. E	207.03 \pm 8.77 ^b	16.62 \pm 0.66 ^b	96.01 \pm 2.94 ^b	18.15 \pm 4.74 ^a ^b
7 th Day	Control	246.25 \pm 3.16 ^a	22.93 \pm 1.11 ^a	113.15 \pm 1.51 ^a	6.15 \pm 0.10 ^b
	Vitamin E	245.97 \pm 1.54 ^a	23.81 \pm 0.78 ^a	113.37 \pm 1.88 ^a	6.15 \pm 0.12 ^b
	Pantoprazole	206.45 \pm 4.29 ^b	16.48 \pm 2.05 ^c	97.28 \pm 2.94 ^b	16.96 \pm 4.14 ^a
	Pantoprazole + Vit. E	226.48 \pm 13.01 ^b	20.41 \pm 1.30 ^b	103.13 \pm 5.73 ^b	10.85 \pm 1.33 ^a ^b
14 th day	Control	249.29 \pm 3.36 ^a	24.54 \pm 1.82 ^a	116.67 \pm 1.60 ^a	6.24 \pm 0.32 ^b
	Vitamin E	249.87 \pm 3.52 ^a	24.69 \pm 0.94 ^a	115.94 \pm 1.30 ^a	6.02 \pm 0.05 ^b
	Pantoprazole	220.98 \pm 1.20 ^b	19.67 \pm 1.66 ^b	104.34 \pm 0.38 ^b	12.00 \pm 1.58 ^c
	Pantoprazole + Vit. E	236.02 \pm 9.65 ^b	21.40 \pm 1.24 ^b	110.54 \pm 4.24 ^a	8.85 \pm 1.54 ^a ^b
21 st day	Control	253.14 \pm 5.59 ^a	23.67 \pm 0.58 ^a	119.93 \pm 1.20 ^a	6.54 \pm 0.39 ^a
	Vitamin E	254.39 \pm 2.12 ^a	24.40 \pm 0.82 ^a	117.84 \pm 4.10 ^a	6.70 \pm 0.49 ^a
	Pantoprazole	238.23 \pm 1.57 ^b	21.47 \pm 0.97 ^b	118.04 \pm 0.91 ^a	8.33 \pm 0.99 ^a
	Pantoprazole +Vit. E	251.94 \pm 6.36 ^a	23.48 \pm 0.26 ^a	118.11 \pm 4.73 ^a	7.21 \pm 0.73 ^a

Values within the same column carrying different subscripts are significantly different at p<0.05.

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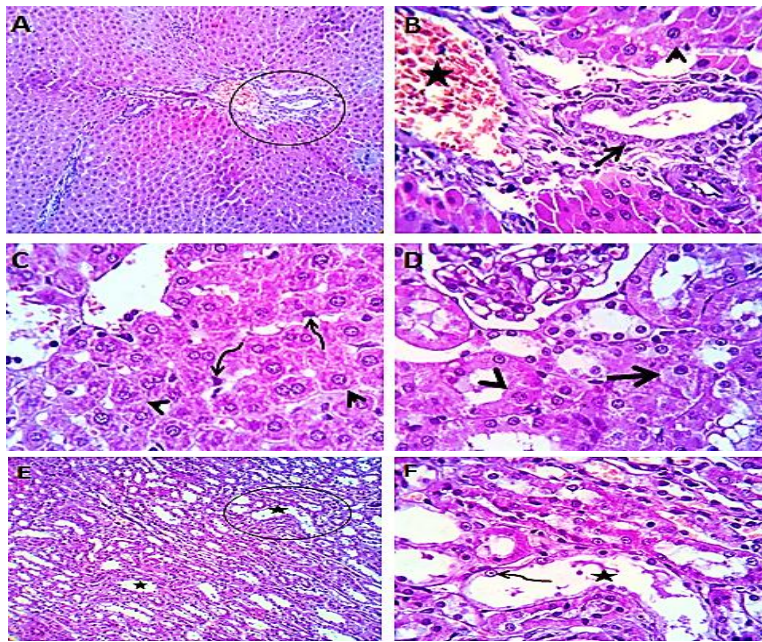


Fig.1: photomicrograph of Liver (A, B&C) showing mild congestion of the portal blood vessels (**star**) with biliary hyperplasia(**arrow**), degenerative changes in some hepatocytes (**arrow heads**) and hypertrophied kupffer cells (**curved arrows**). Kidney (D, E &F) showing degenerative changes in the cortical renal tubular epithelium mostly cloudy swelling (**arrowhead**) and hydropic degeneration (**arrow**) beside dilated collecting tubules (**stars**)with partially atrophied lining epithelium (**curved arrow**). H&E X 100,400.

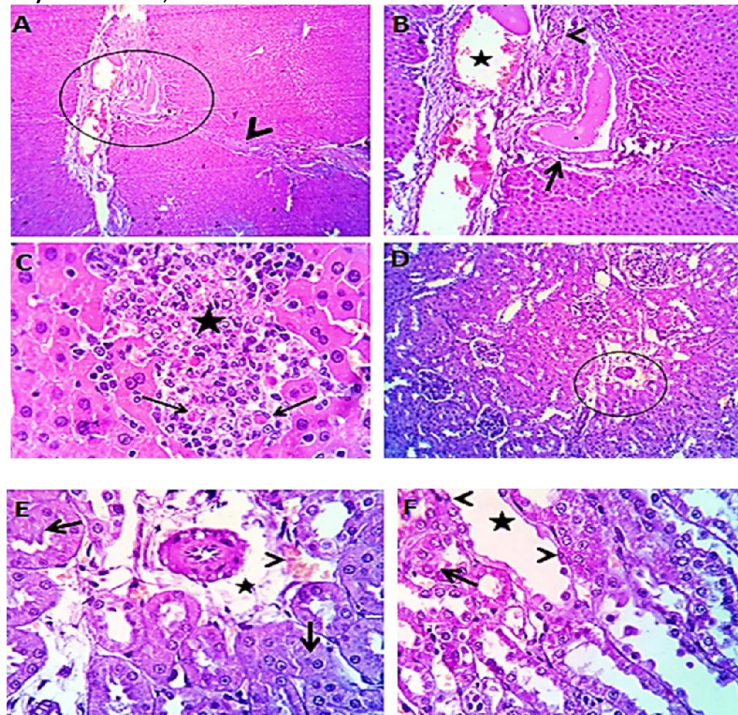


Fig.2: photomicrograph of liver (A, B) showing characteristic biliary hyperplasia (**arrow**)with periductal fibroblast and extension of the reaction to connect adjacent portal triads (**arrowhead**). (C) Moderate interstitial round cells aggregations (**star**)with apoptotic hepatocytes in between (**arrows**). Kidney (D & E) showing mild perivascular edema(**star**), degenerative changes in the renal tubular epithelium(**arrows**)and congestion of intertubular capillaries (**arrow head**) beside (F) dilated renal tubules(**star**) and atrophied renal epithelium (**arrow heads**). H&E X 100,400.

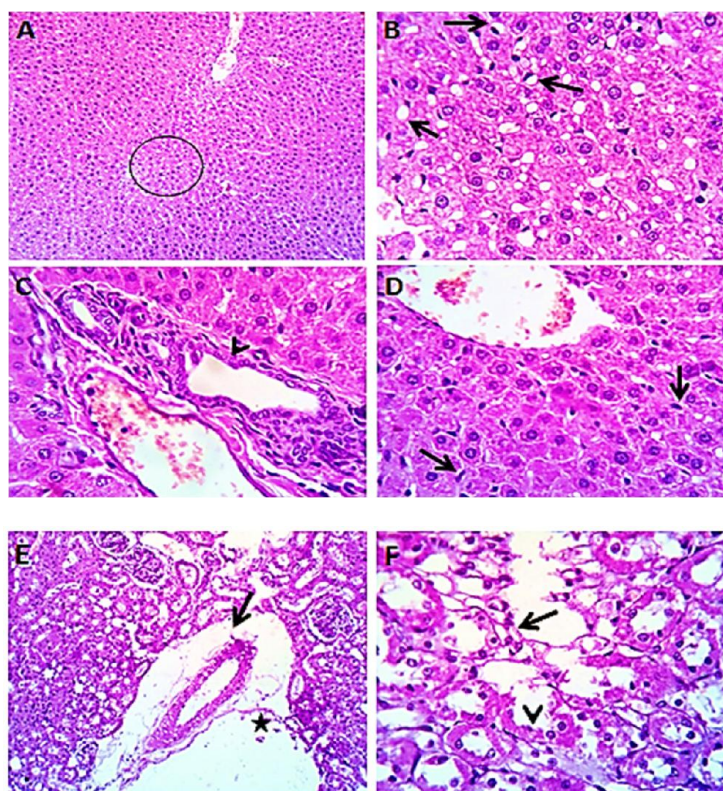


Fig.3: Photomicrograph of liver (A, B & C) showing apparently normal hepatocytes with fatty changes in some hepatic cells (**arrows**) and the portal area show mild biliary proliferation (**arrowhead**). (D) The kupffer cells are hypertrophied (**arrows**). Kidney showing (E) congested renal blood vessels (**arrow**) with perivascular edema (**star**). (F) The renal tubular epithelium showing degenerative (**arrow**) and necrotic changes (**arrowhead**). H&E X 100,400.

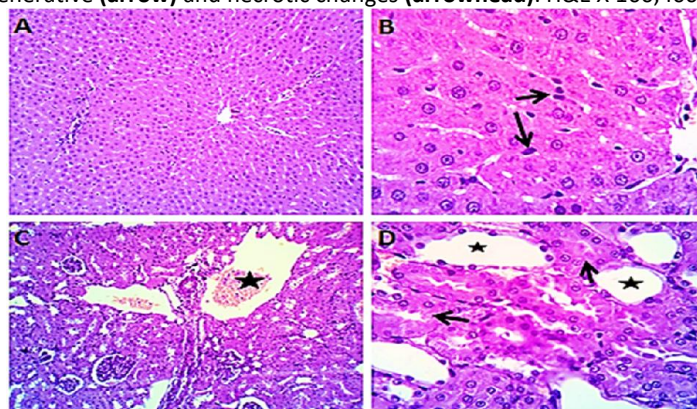


Fig.4: photomicrograph of liver (A & B) showing normal hepatic parenchyma with hypertrophied kupffer cells (**arrows**). Kidney (C) showing mild congestion of renal blood vessels(**star**), (D) degenerative changes (**arrows**) and dilated medullary collecting tubules (**stars**). H&E X 100,400.

4. DISCUSSION

Toxic effects of reactive oxygen species (ROS) could be overcome by cells in different mechanisms, as it scavenges the free radicals inside the cell and terminate the chain reaction (Proctor and Mc Guinness 1986).

Free radicals cause deleterious effect to cellular and intracellular structures, so administration of antioxidants prevent oxidation of fatty acid in the cell (Droke and Loerch, 1989). Antioxidants can inhibit or delay the oxidation of susceptible cells so that,

prevent occurring of oxidative stress (Rice-Evan et al., 1996).

Chemical agents are known to induce hepatic and renal disturbance in human. Vit. E. is considered as one of the most important antioxidants due to its hepato-nephroprotective properties as reported by Pryor et al. (1993). This study demonstrated the hepato-nephroprotective effect of vit. E as it scavenges the free radicals inside the cells, so act as a membrane stabilizer. It decreases degenerative effect of free radicals in liver tissue (Bradford et al., 2003). Another paper by Arbid et al., (2000) stated that vitamin E has been shown to suppress chemically induced carcinogenesis in some animal studies. The anti-oxidative tocopherols are important not only for limiting tissue damage, but also in preventing increases in cytokine production, and also excreting anti-inflammatory effects in man and animals (Bland, 1998).

Chance et al., (1997) mentioned that a reduction in the activities of CAT and SOD by the drug may render the liver more susceptible to hydrogen peroxide (H_2O_2) and hydroxyl radical- induce oxidative stress. Cejkova et al., (2001) reported that a decrease in activity of SOD might increase the cell susceptibility to attack by O_2 . Catalase catalyzes the breakdown of H_2O_2 generated by the action of SOD to water and O_2 . These previous studies explained the hepatoprotective effects of Vit. E which observed in the present study.

Vitamin E reduces levels of catalase, superoxide dismutase, glutathione peroxidase, malondialdehyde and improved histopathological changes occur in the liver and kidney lesions induced by pantoprazole administration. The possible pathway can be explained through chemical structure of vit. E. In this study, vit. E used in a dose (100mg/kg

orally, once daily) for 21 days to clarify the hepatic-nephroprotective effect on rats. We observed a significant rise in the activities of anti-oxidative enzymes and a significant decrease in MDA activity.

Histopathological results of liver sections on the 7th day showed the portal area with mild biliary proliferation and the kupffer cells were mildly hypertrophied. On the 14th day showing normal hepatic parenchyma portal structures and vascular tributaries a few hepatocytes showed cloudy swelling and the kupffer cells were mildly hypertrophied. Kidney on 7th day showing the renal blood vessels were mildly congested with perivascular edema. On the 14th day showing mild congestion of renal blood vessels and dilated medullary collecting tubules with partial atrophy of the lining epithelium than that observed by administration of pantoprazole.

Sachnez-Valle et al. (2012) reported that the anti-oxidative therapy considered as a good cure approach for liver diseases. Medina and Moreno-Otera (2005) reported that vitamin E has a beneficial effect in treatment of liver diseases. Parola et al. (1992) reported that vit. E reduces liver damage in rats. Beytut et al. (2003) reported that the increase in level of antioxidant enzymes like CAT, SOD and GPX resulted from vit. E administration might terminate the peroxidation of lipids and histopathological changes inside the cell, so prevent oxidative damage which caused from cytotoxic drugs.

Kagan (1989) reported that α -tocopherol was believed to protect cell membrane from oxidation of lipid components rendering the membrane more stable as it observed by reduction in free radical species. It has been clarified by Halliwell and Gutteridge (2002) that vitamin E prevent oxidative damage

through scavenging of lipid peroxide radicals before attacking the membrane lipids.

5. Conclusion

It could be concluded that pantoprazole has unpleasant side effects, like any other chemical compound, The PPI agent causes oxidative stress and some histopathological alterations in liver and kidney of rats. Vitamin E has a protective effect against pantoprazole side effects indicating that the drug produced its side effects via free radical formation and by inhibition of the antioxidant systems. The administration of vit. E to pantoprazole should be prescribed together to get optimum pantoprazole results.

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