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Protective Effect of Silymarin and Vitamine-E in Hepatotoxicity Induced by Valporic Acid in Albino Rats

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Administration of valporic acid, the most common medication prescribed against epilepsy produces many metabolic and morphological aberrations in liver due to the fact that liver is the main detoxifying site for these antiepileptic drugs. This experimental work was done for study of the prophylactic role of silymarin and vitamin E in hepatotoxic induced by valporic acid in albino rats. Fifty adult male albino rats weighting 150-200 g were divided into five equal groups, one control and the other four for the drugs. Group I control group is subdivided into two subgroups (IA, IB). Group II ingested valporic acid, group III ingested valporic acid+silymarin, group IV ingested valporic acid+vitamine E and finally group V ingested valporic acid+both drugs, the ingestion was done through orogastric tube for four week. After four weeks biochemical studies (ALT, AST and total bilirubin) were done for all rats in all groups, then the rats were sacrificed and histopathological studies were done for their livers. Biochemical analysis revealed significant increased in AST, ALT and total bilirubin in the group II, III, IV and V in comparison with control groups and revealed significant decrease in the group III, IV and V in comparison with group II. Histopathological examination of the group II revealed necro-inflammatory foci with infiltration of the hepatic lobules with inflammatory cells and inflammation in the portal tract. Histopathological examination of the liver section of group III, IV and V showed mild necrosis and inflammation in hepatic lobules and showed mild inflammation in the portal tract. The liver is highly affected by ingestion of valporic acid. However, ingestion of silymarin and/or vitamine E that is naturally occurring antioxidants can decrease this harmful effect of these drugs on the liver. Therefore, the patient on chronic use of valporic acid must use silymarin and/or vitamin E to protect their livers.

Key words: Silymarin, vitamine E, valporic acid, AST, ALT, liver histopathology

INTRODUCTION

Epilepsy being an important problem from a medical, social and legal point of view is the third most common serious neurological disorder following stroke and Alzheimer's disease (Kammerman and Wassermann, 2001; Villanueva-Gomez and Fernandez-Miranda, 2002).

Valporic acid is an antiepileptic drug and has been widely used for clinical purposes since 1978 (Dickinson *et al.*, 1979). Valporic acid is a branched carboxylic acid and is an established broad-spectrum antiepileptic drug (DeVane, 2003; Henry, 2002). It is only drug capable of controlling all types of seizures associated with the idiopathic generalized epilepsies (Mattson *et al.*, 1992).

Hepatitis more frequently observed in patients taking valporic acid (Gopaul *et al.*, 2003). Toxic effects of valporic acid by which free radicals have role in cytotoxicity, hepatotoxicity (Graf *et al.*, 1998; Jurima-Romet *et al.*, 1996). It is known that free radical reduction lead to decrease in body antioxidant defense and increase in production of free radicals. In addition, it also induces oxidative stress (Durand *et al.*, 1997; Jaeschke, 2011).

Oxidative stress is involved in the release of pro-inflammatory mediators (cytokines and chemokines) and injury to the liver cells (Ha *et al.*, 2010; Jaeschke, 2011). Silymarin, a mixture of flavonolignans isolated from *Silybum marianum* (milk thistle), has been used to treat liver diseases for hundreds of years (Pradhan and Girish, 2006; Saller *et al.*, 2009). The structural similarity of silymarin to steroid hormones is believed to be responsible for its protein synthesis facilitatory actions (Saller *et al.*, 2001).

The hepatoprotective effect of silymarin is mainly due to its strong antioxidant activity and scavenging the free radicals (Tsai *et al.*, 2008). It also effectively inhibits the inflammation (Puerta *et al.*, 1996; Katiyar, 2005) which can also damage the liver (Luster *et al.*, 2001). Silybin is an effective antioxidant, conserving GSH in liver cells while stabilizing the liver cell membranes against oxidative attack (Kosina *et al.*, 2002; Shaker *et al.*, 2010).

Vitamin E generally functions as an antioxidant by reacting directly with reactive oxygen intermediates and has a vital role in defenses against oxidative stress and harmful effects of molecules which chemically destroy fat, protein and DNA (Al Deeb *et al.*, 2000).

The aim of this work is to study the prophylactic effect of silymarin and/or vitamin E in hepatotoxic induced by valporic acid in albino rats.

MATERIALS AND METHODS

Materials

Animals: Fifty adult male albino rats weighting 150-200 g were included in this study. They were divided into 5 equal groups, one control and the other 4 for the drugs. These drugs were given by oral ingestion through an orogastric tube. After four weeks, blood samples were taken from inner canthus of the eye by capillary tubes for measurements of serum alanine transaminase (ALT), serum aspartate transaminase (AST) and total bilirubin. Then all rats of the groups were scarified and dissected for light microscopic examination of their livers.

Drugs:

- **Valporic acid:** It was obtained in the form of valporic acid 200 and 500 mg, tablets, valporic acid, produced by Global Napi Pharmaceutical Industries Company
- **Silymarin:** It was obtained from SEDICO pharmaceutical a company in the form of sachets, each contains 140 mg of silymarin
- **Vitamin E:** It was obtained from Arab Company for Pharmaceutical and Medicinal Plants (MEPACO), Egypt, in capsular form. Each capsule contains 400 mg, equal to 400 IU (Alpha tocopherol acetate)

A Valporic acid and silymarin drug was suspended in 5.0 mL of deionized water, while vitamin E was dissolved in corn oil in accordance to Onyema *et al.* (2006). All preparations and appropriate concentrations were prepared with the help of a biochemist.

Grouping of animals: Fifty male albino rats were divided in five equal groups as follows:

- **Group I: Control group:** Included 10 rats on standard diet and deionized water (freely given). They were divided into two subgroups:
 - **Subgroup IA (Negative control group):** Included 5 rats on usual diet and had no any addition of medications or drugs
 - **Subgroup IB (Positive control group):** Included 5 rats on usual diet and 0.5 mL of corn oil (vehicle), to test if it has any effects on the rat liver cells
- **Group II: Valporic acid treated group:** Included 10 rats which were treated with valporic acid in 300 mg kg⁻¹ day⁻¹ for four week orally in accordance to Baran *et al.* (2004)

- **Group III:** Included 10 rats which were treated with valporic acid in 300 mg kg⁻¹ day⁻¹ plus silymarin 50 mg kg⁻¹ day⁻¹ for four week orally in accordance to Girish and Pradhan (2012)
- **Group IV:** Included 10 rats that were treated with valporic acid in 300 mg kg⁻¹ day⁻¹ plus vitamin E 100 mg kg⁻¹ day⁻¹ for four week orally in accordance to Tayal *et al.* (2007)
- **Group V:** Included 10 rats which were treated with valporic acid in 300 mg kg⁻¹ day⁻¹ plus silymarin 60 mg kg⁻¹ day⁻¹ and vitamin E 100 mg kg⁻¹ day⁻¹ for four week orally

Method

Anesthesia: After four week of treatment, the animals were deeply anesthetized by ether inhalation in a ball jar. Then, blood samples were collected for different laboratory analysis from inner canthus of the eye by capillary tubes.

Biochemical parameters studied: Blood samples of about 5 cm were obtained for biochemical analysis after four weeks of ingestion of the drugs from all rats.

The following biochemical parameters were used for each sample:

- **ALT:** Kinetic method was used. The enzymatic tests were performed on Architect CI-4100 purchased from Abbot Company
- **AST:** Kinetic method was used. The enzymatic tests were performed on Architect CI-4100 purchased from Abbot Company
- **Total bilirubin:** Kinetic method was used. The enzymatic tests were performed on Architect CI-4100 purchased from Abbot Company

Histopathological study: After four weeks of daily ingestion of the drugs, all rats of each group were anaesthetized then sacrificed by cervical dislocation. After scarification livers were carefully dissected from each rat and were prepared for histological examination according to Carleton *et al.* (1980).

Statistical analysis: Data analyzed by software statistical computer package (SPSS) version 13. Paired student's (t) test was employed for comparison between groups. Difference was considered significant at p<0.05 level. All values were expressed as Mean±SD.

RESULTS

In the present study, there was no statistically significant difference between (IA) negative control and (IB) positive control subgroups. Thus, in the

subsequent results comparison referred as a control group. Results of biochemical study are shown in Table 1.

Results of histopathological study by light microscopically examination of the liver section of the different groups:

- **Group I:** Showed no abnormal histopathological changes. They contained large number of hepatic lobules. The hepatic lobules were roughly spherical in shape with a venous channel and a cortical vein. Irregular interconnecting sheets or plat-like arrangements radiated outwards from the central vein and constituted the parenchyma of the lobule. Sinusoids separated the sheets of hepatic cells. At the peripheral angle of each lobule, the portal area contained branches from the portal vein, hepatic artery and bile duct (Fig. 1)

Table 1: Statistical comparison between studied groups as regard serum AST, ALT and serum bilirubin

Parameters	Mean	SD	t	p
AST (U L⁻¹)				
Group 1 (control)	23.80	4.42	0.97	0.365
Group 2 (valporic acid)	64.60	8.54	9.68	0.000****
Group 3 (valporic acid+silymarin)	44.48	4.17	2.93	0.012*
Group 4 (valporic acid+vitamin E)	49.71	5.83	3.44	0.021*
Group 5 (valporic acid+both drugs)	43.89	4.45	3.02	0.016*
ALT (U L⁻¹)				
Group 1 (control)	18.34	2.01	1.76	0.171
Group 2 (valporic acid)	44.45	2.50	12.35	0.000****
Group 3 (valporic acid+silymarin)	29.03	3.11	8.57	0.029*
Group 4 (valporic acid+vitamin E)	33.49	2.57	16.09	0.039*
Group 5 (valporic acid+both drugs)	21.30	1.16	16.18	0.019*
Serum bilirubin (mg dL⁻¹)				
Group 1 (control)	0.365	0.1289	0.12	0.732
Group 2 (valporic acid)	1.07	0.18	9.56	0.000****
Group 3 (valporic acid+silymarin)	0.69	0.07	2.64	0.036*
Group 4 (valporic acid + vitamin E)	0.77	0.08	2.15	0.035*
Group 5 (valporic acid + both drugs)	0.51	0.0915	2.64	0.044*

M: Mean, SD: Standard deviation, *: Significant, ****: Means very highly significant

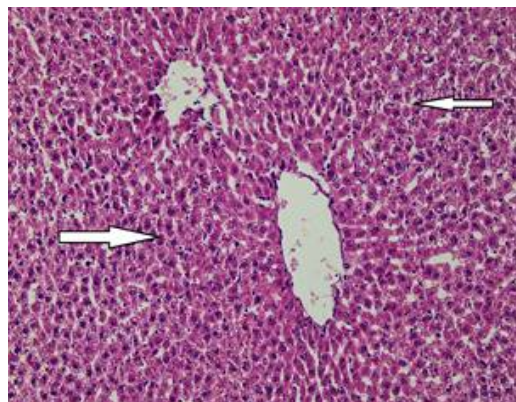


Fig. 1: Photomicrograph of liver tissue from control group (H and E) x 200

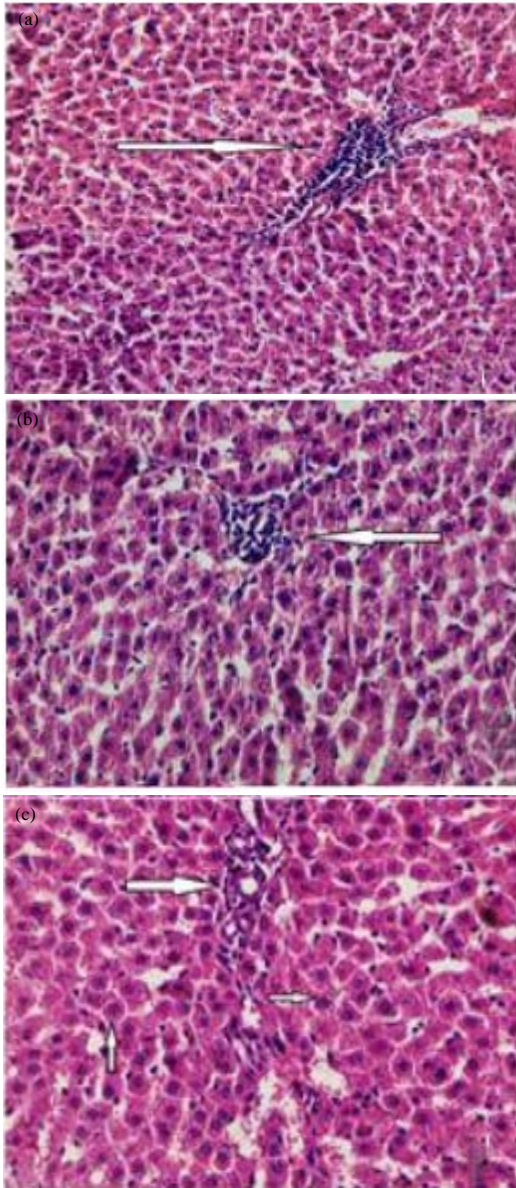


Fig. 2(a-c): Photomicrograph of the liver of group IV: (a) Portal triaditis, (b) Hepatic cells infiltrated with inflammatory cells and (c) Hepatic cells with nuclear degeneration with prominent nucleoli and inflammation of the portal tract (H and E) x 200

- **Group II:** Showed necroinflammatory foci with infiltration of the hepatic lobules with inflammatory cells. The portal tract showed inflammation (portal triaditis) (Fig. 2)
- **Group III, IV and V:** Showed mild necrosis and inflammation in hepatic lobules and showed mild inflammation in the portal tract (Fig. 3)

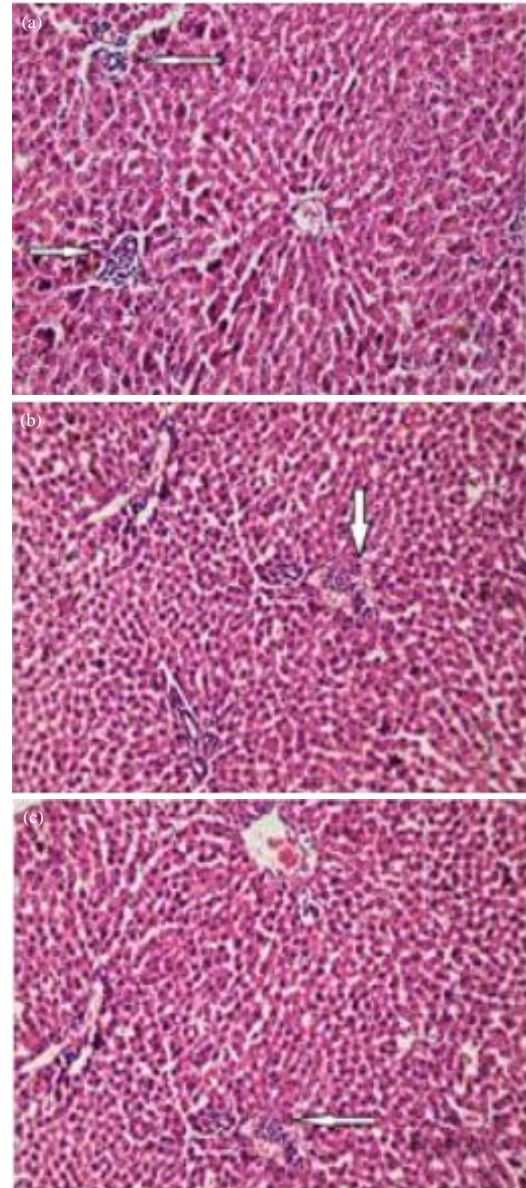


Fig. 3(a-c): Photomicrographs of livers of: (a) Group V: Mild portal triaditis and hepatic cells infiltrated mildly with inflammatory cells, (b) Group VI: Hepatic cells infiltrated mildly with inflammatory cells and (c) Group VII: Hepatic cells infiltrated mildly with inflammatory cells (H and E) x 200

DISCUSSION

Hepatitis more frequently observed in patients taking valporic acid than other antiepileptic drugs (Gopaul *et al.*, 2003).

In the present study, there was no statistically significant difference between (IA) negative control and (IB) positive control subgroups, the histopathological studies of the liver of these groups show normal hepatic lobules and sinusoid and normal bile tract. AST, ALT and total bilirubin of valporic acid group were very highly significant increased in comparison with the control group. These results were in accordance with Tong *et al.* (2003), who said that these valporic acid drug induce hepatotoxicity by a multiple step mechanism. The exact mechanism responsible for liver injury caused by these drugs remains unknown. It may hypothesize to involve the generation of toxic metabolites and/or reactive oxygen species. The reactions of toxic metabolites with glutathione in mitochondria produce a localized depletion of glutathione that would result in oxidation stress. Oxidative stress precedes the onset of steatosis and necrosis in liver.

Jurima-Romet *et al.* (1996) and Graf *et al.* (1998) said that toxic effect of valporic acid by which free radicals have role in cytotoxicity, hepatotoxicity. It is known that free radical reduction lead to decrease in body antioxidant defense and increase in production of free radicals. In addition, it also induces oxidative stress Tang *et al.* (1997) found that rats show a similar genetically determined valporic acid mediated oxidative damage are generally attributed to the formation of free which act as stimulator of lipid peroxidation and source for destruction and damage to the cell membrane.

Alterations of various cellular defense mechanisms consisting of enzymatic and non-enzymatic components (reduced glutathione) have reported in valporic acid-induced hepatotoxicity (Tong *et al.*, 2005). These results were in accordance with Tong *et al.* (2003), said that oxidative stress is one of the mechanisms for valporic acid-induced hepatic injury. Majority of normally formed free radicals removed by the action of reduced glutathione. In circumstances where there is a reduction in glutathione results in the initiation of lipid peroxidation resulting in tissue injury. Histopathological examination of the liver shows necro-inflammatory foci in the hepatic lobules and inflammation in the portal tract (portal triaditis).

Khan *et al.* (2011), who reported that histopathological changes in animals receiving valporic acid was portal inflammation, fatty changes and liver cell necrosis.

Studying valporic acid+silymarin group, AST, ALT and total bilirubin of this group were very highly significant increased in comparison with the control group and were significantly decreased in comparison with the valporic acid group. Histopathological examination of the group liver show mild degree of necrosis and mild inflammation in the portal tract. These results were in accordance with Kosina *et al.* (2002), who said that; the preclinical studies using different hepatotoxic substances

showed that silymarin has multiple actions as a hepatoprotective agent. The antioxidant property and cell-regenerating functions because of increased protein synthesis considered as most important. It provides an important additional protection against oxidative damage. There are varieties of antioxidants in silymarin which protect against disease-causing oxidative damage.

Non-protein thiol is an important defense mechanism in living cells. As a substrate for antioxidant enzymes, i.e., glutathione peroxidase and glutathione reductase, it protects cellular constituents from the damaging effects of peroxidase formed in metabolism and other reactive oxygen species reactions. Aged garlic extract increases cellular glutathione in a variety of cells including those in normal liver and mammary tissue (Tanaka *et al.*, 2004).

AST, ALT and total bilirubin of valporic acid+vitamin E group were highly significant increased in comparison with control group and were significantly decreased in comparison with valporic acid group. Histopathological examination of group livers shows mild degree of necrosis and mild inflammation in the portal tract. These results were agree with Aluclu *et al.* (2009), who reported that chronic treatment with valporic acid decrease antioxidant level of vitamin E. The related studies have shown that vitamin E has a protection effect against hepatotoxicity resulting from valporic acid application. Vitamin E is a natural antioxidant and extremely effective in detoxifying harmful free radicals in various tissues. It has inhibited hepatocytes lipid peroxidation caused by free radical forming agents and this reflects the ability of tocopherol to scavenge reactive species within the lipid region of the membrane and thereby, prevent cell injury (Tayal *et al.*, 2007). Buchi *et al.* (1984) has revealed that antioxidant containing α -tocopherol and N, N diphenyl-p-phenylenediamine have been effective in reversing the hepatotoxicity induced by valporic acid in rats. Vitamin E, the major lipid-soluble antioxidant in liver, can scavenge free radicals and preserve membrane integrity. It protects cells and sub-cellular structures from the oxidative damage by decreasing LPO products (Bansal *et al.*, 2005).

Sodergren *et al.* (2001) said that; antioxidants recognized to scavenge free radicals, might prevent propagation of the drug-induced lipid peroxidation process. Vitamin E is a well-characterized chain-breaking antioxidant with the particular function of preventing lipid peroxidation in membrane systems.

Continuous valporic acid treatment decrease level of antioxidants such as vitamin E and glutathione peroxidase, resulting in oxidative stress (Tamai *et al.*, 1988; Kotch *et al.*, 1995; Amel *et al.*, 2012).

In short, the liver is highly affected by ingestion of valporic acid. However, ingestion of silymarin and/or vitamin E that is naturally occurring antioxidants can

decrease this harmful effect of these drugs on the liver. We recommended that the patient on chronic use of valproic acid must do periodic investigation of liver function and they must use silymarin and/or vitamin E to protect their livers.

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